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THE COMPOSITION OF CRUDE GLUTEN¹

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Introduction

It is a matter of common observation that crude gluten contains only 75-80% protein. As scant attention has been paid to the 20-25% of non-protein constituents of gluten, it has seemed worth while to investigate the nature of these substances. While the assumption has commonly been made that proteins and inorganic salts determine gluten quality, it seems irrational to rule out the influence of the other constituents of gluten until they are more definitely identified. It is conceivable that a favorable influence might be exercised by some of these constituents and an unfavorable one by others.

The literature furnishes surprisingly little information about the non-protein constituents of gluten. Von Bibra (1860) noted the presence of fat in gluten, and Ritthausen (1864) found both fat and starch in gluten. Wanklyn and Cooper (1881) report that dry gluten contains 1% fat and 0.3% ash on the flour basis, which indicates that gluten itself contains about 10% fat and 3% ash. Osborne and Voorhees (1893) noted in the preparation of glutenin from a straight flour from spring wheat "the presence of gummy matter." They found that gluten washed free from gliadin with alcohol (sp. gr. 0.90) did not wholly disperse in 0.15% KOH solution. They rejected the sediment and precipitated the dispersed material by the addition of a suitable amount of HCl. This precipitated glutenin could be purified only by repeated washing with

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alcohol and ether. Most glutenin preparations of earlier investigators, they pointed out, had been incompletely purified at this point. They obtained in the ether washings "a considerable amount of phytocholesterin and lecithin together with fat. It is probable that all these bodies are held suspended in solution by aid of the dissolved proteids as an emulsion."

Working (1924) concludes that lipoids influence gluten quality. His interpretation of the word lipoids is similar to that of Thatcher (1921) but not in harmony with the broader meaning given this term by Czapek (1913), whose theory of lipid distribution in the plant cell (1919) is discussed by Working. Czapek's interpretation is essentially that agreed upon by Bang (1911), Bloor (1920), Mathews (1920), and MacLeod (1922). Working found that the addition of phosphatides "to flour in small quantities injured gluten quality as measured by the feel of the hand-washed gluten, by viscosity as measured by the MacMichael viscosimeter, and by baking tests." His observations on the effect of soap on gluten quality may be explained in part by the associated decrease in the hydrogen-ion concentration. The bearing of his investigations is likely to be of great significance in connection with the variable effect of different shortenings. It is interesting to note that the color of gluten is frequently related to gluten quality. The constituents responsible for this color are ether-soluble.

Exact methods of determining lipid- P_2O_5 in flour, pastes, etc., have been developed recently by Jacobs and Rask (1921) and by Hertwig (1923a, 1923b). Hertwig's acid digestion method (1923a) is similar in principle to the familiar Schmidt-Bondzynski method for fat in cheese. Hertwig's neutral extraction method (1923b) consists in preliminary treatment with hot dilute alcohol followed by extraction with ethyl and petroleum ethers. On the basis of a limited number of observations, Hertwig (quoted by Buchanan, 1924) concludes that flour, dry basis, contains an average of 0.055% of lipid- P_2O_5 . On the basis of a distearyl lecithin, this is equivalent to 0.63% phosphatides in flour. This may be considered a minimal figure, for the phosphatides prepared from wheat flour by Winterstein and Hiestand (1908) and by Winterstein and Smolenski (1909) contained less phosphorus than distearyl lecithin contains.

In the most complete analysis of gluten on record, Norton (1906) found 9.44% carbohydrates other than fiber, 4.20% ether extract, 2.02% fiber, and 2.48% ash.

Dill and Alsberg (1924) showed starch to be a normal constituent of gluten, for even after one hour's washing, the gluten wash water was somewhat turbid and gave a test for starch.

After washing gluten for a long time, Gerum and Metzger (1923) obtained a gluten which they believed to be starch-free. In computing protein they employed the factor 6.25 instead of the correct factor for protein in flour, 5.7, which has been established by the classic investigations of Osborne. Calculating their data to the dry basis and using the factor 5.7 for protein we obtain: protein 89.8%, ether extract 0.26%, crude fiber 0.58%, and P_2O_5 , 0.35%. Obviously about nine per cent of some unidentified material is present.

That fiber exercises an unfavorable influence on gluten quality seems to be a common conclusion. Thus Lindet and Ammann (1905) state that a coherent mass of gluten can not be obtained from whole meal flours, due, they believe, to greater acidity, to the presence of a mucilagenous substance, to a larger glutenin-gluten ratio, and to the mechanical effect of the bran in preventing agglutination. While Norton (1906) experienced no great difficulty in determining the gluten by the washing process in whole-wheat meal, he agrees that the bran more or less affects the agglutinating power of the gluten. Norton noted that gluten is very tenacious in retaining fiber. His data indicate that nearly all the fiber of flour appears in the gluten.

Briefly, it may be said that gluten contains notable amounts of lipoids, fiber, ash, and carbohydrates easily hydrolyzable by acids. The findings of Gerum and Metzger (1923) suggest that there may be a considerable quantity of some carbohydrate other than starch. Working's study (1924) suggests that a variable amount of phosphate is included in gluten and indicates that gluten quality is modified thereby.

During an investigation of the gluten-washing process (Dill and Alsberg, 1924) several questions arose which remained unanswered because of the paucity of information concerning the nature and concentration of the non-protein constituents of gluten. Accordingly an investigation of this subject has been carried out. The very recent publication of Working's preliminary investigation (1924) on a closely related subject suggested that duplication of effort might be avoided by publication of the data so far obtained.

Experimental

Our first investigation was concerned with the preparation and identification of the carbohydrate material of gluten. Following

one of the procedures of Osborne and Voorhees (1893), 2000 grams of Kansas Kharkoff flour was doughed with tap water and after one hour the gluten was washed out with tap water. (The standard sodium phosphate solution recommended for gluten washing (Dill and Alsberg, 1924), was not employed in this investigation because phosphorus determinations in the dry glutens were anticipated.) The resulting gluten was passed through an Enterprise grinder and was then freed from gliadin by five successive alcoholic extractions at room temperature. An amount of alcohol sufficient to make two liters of solvent containing 60% alcohol by volume was used for the first extraction and two liters of 60% alcohol was used for subsequent extractions. Forty-eight hours or more was allowed for each extraction. The residue, washed free from alcohol, was disintegrated by digestion for four days at room temperature with four liters of 0.2% KOH solution. The supernatant liquid, after separation from the insoluble residue by centrifugation, was treated cautiously with dilute HCl until precipitation took place. The precipitate thus obtained has been shown by Osborne's work to consist chiefly of glutenin and lipoids. Pure glutenin can be obtained from it by repeated extraction with alcohol and with ether. The clear filtrate from the glutenin-lipoid precipitate was found to contain little besides protein and inorganic salts (chiefly KCl). The protein appeared to be globulin, for it was readily soluble in very dilute salt solution, insoluble in 60% alcohol, and only slowly coagulated by boiling. Norton (1906) found that gluten contained globulin.

Evidently the carbohydrates of this gluten were either extracted with 60% alcohol or appeared in the residue which was not dispersed by the dilute KOH solution. Walton and Coe (1923) have shown that the products (including dextrans) obtained by maltase hydrolysis of starch are soluble in 60% alcohol. If any dextrin or sugar did occur in this gluten, it was probably removed during gliadin extraction. No investigation was made of this point.

Other carbohydrate substances such as starch, gums, pentosans, etc., are in general insoluble in 60% alcohol and must therefore be found in the residue which was not dispersed in 0.2% KOH solution. This residue was re-extracted at room temperature with 250 cc. of 0.2% KOH solution, centrifuged, and the process repeated once more. It was noted that a separation into two layers took place during centrifugation. The lower layer was white, compact, and typical of starch. The upper layer, of about equal bulk, was somewhat flocculent and gray in color. After the second centrifugation

gation, the upper layer was transferred to another 100 cc. centrifuge tube with a spoon. These separate portions were thoroly stirred with 75 cc. portions of the 0.2% KOH solution, centrifuged, a more complete separation was made, and the same process was repeated. The separated portions were washed four times with 90% alcohol, separating each time by centrifugation. A final washing with absolute alcohol followed. The composition of these products is shown in Table I.

TABLE I

COMPOSITION OF GLUTEN FRACTION INSOLUBLE IN ABSOLUTE ALCOHOL, 70% ALCOHOL, AND NOT DISPERSED BY 0.2% KOH SOLUTION
(Results calculated to the dry basis)

	White, compact portion (21.8g = 8.2% dry gluten) Per cent	Gray, flocculent portion (7.3g = 2.7% dry gluten) Per cent
Total protein ($N \times 5.7$).....	0.20	6.20
Lipoids	1.00	0.90
Ash	2.30	4.30
P ₂ O ₅	0.09	0.90
Starch (pentosans possibly included).....	93.20	84.30

Nitrogen was determined by the Kjeldahl-Gunning method; lipoids, by the Hertwig acid digestion method (1923a); ash, by the Hertwig-Bailey method (1924); P₂O₅, by the volumetric molybdate method (after preparation of a two-gram sample by treatment with 5 cc. of 10% magnesium nitrate solution, evaporation to dryness, ashing, and solution of the ash in dilute nitric acid); and starch by a modified Pflüger's glycogen method. The Pflüger method was carried through as usual up to the filtration of the starch which had been precipitated in alcohol. Treatment of the starch with boiling water gave too viscous a product to permit filtration. The paper was therefore perforated and its contents washed into a volumetric flask with boiling water. After the usual acid hydrolysis, the solution was nearly neutralized, made up to volume, and sugar was determined by the Munson-Walker method. The cuprous oxide was determined by the volumetric thiosulphate method. This method possesses distinct advantages in the case of gluten, to which it was also applied, for removal of proteins by precipitation is avoided. Pure starch determined by this method gave yields of 98-99% of those obtained by direct acid hydrolysis. Dextrin, gum arabic, and a galactosan (agar agar) were carried through the Pflüger method. Dextrin and gum arabic gave no alcohol precipitate after the digestion with concentrated KOH solution. Agar agar was not disintegrated during the digestion; and after treatment with alcohol, filtration and hydrolysis yielded con-

siderable reducing sugar. The Pflüger method therefore appears to exclude sugars, gums, and dextrans, but includes galactosans and possibly pentosans.

Microscopic examination of the materials whose analyses are shown in Table I, showed little else than typical starch grains. This observation considered in conjunction with the chemical analysis indicates that the white material contained little beside starch, while the gray material consisted largely of starch. The gray material also contains considerable nitrogen and ash. Its high P_2O_5 content may be due either to inorganic phosphates or phytin or both.

The difficulty of Gerum and Metzner in preparing starch-free gluten (1923) led them to the conclusion "beim Anteigen des Mehles auch die Stärke bis zu einem gewissen Masse quillt und in diesem kolloidalen Zustand mit den kleberbildenden Eiweissteilschen zu einem Adsorptionkomplex zusammentritt. Diese Verbindung scheint allerdings nicht das innige Gefüge wie beim Zusammentritt des Gliadins und Glutenins zur Grundlage zu haben, da diese Bindung durch längeres Waschen und Kneten des Klebers lösbar ist."

An attempt was made to determine whether the starch grains of gluten differ in any respect from normal starch grains. The approximate size and relative distribution of small and large starch grains in a flour were determined by microscopic observations. Some of the flour was doughed and the gluten was washed out in the usual way. The wash water was collected in five consecutive portions from the beginning to the end of the washing process. The five lots of starch thus obtained showed no noticeable change from the original in size of grain or in distribution of sizes. Finally the gluten was disintegrated by grinding in a mortar with 60% alcohol. The microscopic appearance of the starch grains thus separated was identical with those obtained above. It would therefore appear that the starch grains are simply imbedded in the protein complex. There was adduced no evidence of their playing any rôle in the gluten complex other than that of an indifferent filler.

Lipoids were next considered. Osborne and Voorhees (1893) reported lecithin in gluten, and this finding has been confirmed by Working (1924). Anderson and Nabenhauer (1924) have found two phytosterols in wheat—sitosterol and dihydrositosterol. Whether or not these appear in the gluten has not been determined.

A preliminary observation on the determination of lipoids may be of interest. A sample of glutenin which had not been washed

with alcohol or ether was dried to constant weight at 100° and atmospheric pressure. Seventy-two hours were required. A portion of this was powdered to pass an 80-mesh sieve. Lipoids were then determined by Hertwig's acid digestion method and "ether extract" was determined by the Soxhlett method, extracting with anhydrous ethyl ether for 12 hours. The former method yielded 11.0% and the latter method 3.1%. The product obtained by the acid digestion method was completely chloroform-soluble. The discrepancy may be due to low initial solubility of the lipoids in anhydrous ether; it may be due to the lipoids having been rendered relatively ether-insoluble by the long drying process, or it may be explained by saponification of the lipoids during the process of extracting the glutenin from the gluten by disintegration in 0.2% KOH (a process which required four days). Obviously the acid digestion method includes soaps (by liberation of the fatty acids) while extraction with a neutral fat solvent does not.

Comparative analyses were then made of two glutes. One of these was a commercial product obtained from the Huron Milling Company. This gluten had been prepared from wheat flour. A mechanical washer was used to separate the starch, after which the gluten was carefully dried at a low temperature. No chemicals or preservatives were employed. Nearly all of it readily passed an 80-mesh sieve.

A second gluten was prepared from an eastern soft red winter flour. The washed-out gluten was rapidly dried in vacuo at $80-90^{\circ}$. It was ground to pass an 80-mesh sieve.

Moisture was determined in the samples by heating to constant weight at 110° and atmospheric pressure. Three hours were required. The other determinations were all calculated to the dry basis. Nitrogen, ash, total P_2O_5 , and starch were determined by the methods described above. Starch was also determined by direct acid hydrolysis. This method quite likely includes in addition to starch, pentosans and possibly other carbohydrates. It was carried out as follows:

A 2-gram sample was weighed into a 200 cc. volumetric flask and mixed with 100 cc. of water and 10 cc. of HCl, sp. gr. 1.125. This was then heated for three hours in a boiling water bath, cooled, nearly neutralized, again cooled, made up to volume, and filtered; 100 cc. of the filtrate was transferred to a 200 cc. flask and precipitated with a 25% solution of phosphotungstic acid. Reducing sugars were determined in the filtrate by the Munson-Walker method. Cuprous oxide was determined by the volumetric thiosul-

phate method. Glucose $\times 0.93$ = starch. Lipoids and lipid- P_2O_5 were determined by Hertwig's neutral extraction method (1923b). Lipoids were also determined by Hertwig's acid digestion method (1923a). Phytin- P_2O_5 was determined by the method of Heubner and Stadler (1914) as applied to plant products by Rather (1917).

The results, reduced to the dry basis, are shown in Table II.

TABLE II
COMPOSITION OF DRY GLUTEN

	Gluten A (from Durum flour)*		Gluten B (from eastern soft red winter wheat flour)		Gluten C (Commercial product)
	% in crude gluten	% recovered in crude gluten from flour	% in crude gluten	% recovered in crude gluten from flour†	% in crude gluten
Protein (N $\times 5.7$).....	80.91	13.39	72.67	9.660	81.00
Ether extract	4.20	0.69	0.75‡
Lipoids (neutral extraction method)	7.05	0.940	11.56
Fiber	2.02	0.34
Ash	2.48	0.41	0.63	0.084	0.91
Carbohydrates (acid hydrolysis)	9.44	1.56	18.82	2.400	4.93
Total.....	99.05	99.17	13.080	98.40
Carbohydrates (Pfaller glycogen method).....			17.52	2.330	3.50
Total P_2O_5			0.38	0.051	0.46
Phytin- P_2O_5			0.05	0.007	0.22
Inorganic P_2O_5 §			0.23	0.031	0.14
Lipoid- P_2O_5			0.10	0.013	0.10
Calculated phosphatide (as distearyl lecithin).....			1.13	0.150	1.13
Lipoids (acid digestion method).....			5.01	0.660	10.76

* Norton's analysis (1906).

† Calculated to the dry flour basis. Norton's corresponding figures are based on air-dry flour of unknown moisture content.

‡ Not included in the total.

§ Calculated by difference.

It is evident from the data shown in Table II that gluten may vary widely in composition. The lipid content may range from 5 to more than 10 per cent; the ash may vary within wide limits; the carbohydrates, shown to be chiefly starch, may vary from 5 to nearly 20 per cent.

In the two glutes examined, the lipid- P_2O_5 contents were about equal. Calculation of the phosphatide as distearyl lecithin indicates a minimum of more than one per cent phosphatide.

Approximately one fourth the total phosphorus of these glutes was lipid phosphorus. This distribution of the remainder between inorganic phosphorus and phytin-phosphorus was found to be quite different in the two glutes. It appears that the commercial gluten contains four times as much phytin as the other gluten. These results must be considered as approximate because of the small

amount of phytin- P_2O_5 present and because the endpoint of the method is somewhat uncertain. It is believed, however, that the results give a true approximation of the distribution of inorganic and phytin- P_2O_5 in these glutes.

The very small amount of ether extract obtained from Gluten B by the Soxhlett method is striking. This suggests that the unexplained feature of the gluten analysis by Gerum and Metzger (1923) was their failure to secure extraction of lipoids with ethyl ether. On the other hand, Norton's figure of 4.2% ether extract, presumably by the Soxhlett method, is unexplained.

It is interesting to note that in each gluten a notably higher content of "lipoids" was obtained by the neutral extraction method than by the acid digestion method. The product in each case was entirely soluble in chloroform. That extracted by the neutral method was not liquid at room temperature, while that extracted by the acid digestion method was entirely liquid. It is possible that the lower values found by the acid digestion method may be due to the splitting off of some of the carbohydrate groups or nitrogen-containing groups which have been shown by Winterstein and Hiestand (1906) to be present in plant phosphatides.

The composition of the flour from which Gluten B was prepared is shown in Table III. The proportion of flour constituents obtained in the gluten is also shown in this table. This flour is the same as that employed for gluten washing in Table XI, Dill and Alsberg (1924).

TABLE III
COMPOSITION OF AN EASTERN SOFT RED WINTER WHEAT FLOUR (DRY BASIS) AND YIELD OF GLUTEN CONSTITUENTS

	Flour per cent*	Percentage of each constituent appear- ing in gluten
Protein ($N \times 5.7$)	11.480	84.5
Lipoids (neutral extraction method).....	1.850	47.5
Ash	0.540	15.6
Total P_2O_5	0.244	20.4
Phytin- P_2O_5	0.090	13.0
Inorganic- P_2O_5 †	0.140
Lipoid- P_2O_5	0.051	26.0

*It is important to note that these figures are calculated to the anhydrous basis. The yield of dry gluten was 11.6% on the fresh flour basis and 13.3% on the anhydrous flour basis. The moisture content of the flour was 12.4%.

†Calculated by difference. Possibly one-third of this fraction is starch- P_2O_5 . Northrup and Nelson (1916) have shown that purified potato starch contains in some form of chemical combination about 0.06% P_2O_5 .

The data of Table III indicate that about half the total lipoids of this flour appeared in the gluten, altho only one-fourth of the lipid- P_2O_5 appeared in the gluten. The small proportion of flour ash remaining in the gluten is also interesting.

Conclusions

The principal carbohydrate of gluten is starch. While the starch content of gluten is widely variable, no evidence was found that it plays any rôle in gluten quality other than that of an indifferent filler.

Results for total lipoids in gluten obtained by Hertwig's procedures were strikingly higher than those obtained by the Soxhlett method using anhydrous ethyl ether.

Analyses of a soft red winter wheat flour and of the gluten obtained from it showed that five-sixths of the total protein, one-half of the total lipoids, one-sixth of the ash, one-fifth of the total P_2O_5 , and one-fourth of the lipoid- P_2O_5 which were present in the flour appeared in the gluten.

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WHEAT AND FLOUR STUDIES, III. THE AMINO NITROGEN CONTENT OF THE IMMATURE WHEAT KERNEL AND THE EFFECT OF FREEZING.

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Introduction

Kedzie (1893), Teller (1898), Nedokutschajew (1902), Brenchley and Hall (1908-10), Thatcher (1915), Swanson, Fitz, and Dunton (1916), and others, have shown that as the normal wheat kernel develops the amount of the simpler nitrogen compounds decreases. In all the cases cited the workers apparently used air dried material.

The findings of Eckerson (1917) who applied microchemical methods to both fresh and dried material, are of special interest in this connection and some of her conclusions are:

"The nitrogen compounds, aside from the aleurone and protoplasm, in the endosperm just before ripening of the grain are: much asparagine, considerable arginine, histidine, and some leucine. No glutemine was found.

"On desiccation of the grain, protein appears in the storage cells; the amino-acids and most of the asparagine disappear. The protein has the physical characters of gluten.

"Formation of the storage protein in wheat seems to be a condensation process, and it takes place on desiccation of the wheat kernel."

Olson (1917) found that the higher the temperature at which immature harvested wheat was dried the less the crude gluten obtained from it and the higher the amide nitrogen content of the resulting flour. He also found that when the harvested wheat stood in the laboratory before subjecting it to heat, the gluten content increased and the amino nitrogen content of the resulting flour decreased. He concludes that, "In the earlier stages of kernel development no gluten is found, . . . that the formation of gluten takes place in the kernel at a time when translocation falls off rapidly. Dessication also sets in at this time."

Shutt (1907) found that frosted wheat contained a larger percentage of its nitrogen in non-albuminoid form than sound wheat. Blish (1920) confirmed Shutt's findings and also showed that the more immature the wheat at the time of frost the higher

¹ Published with the approval of the Director.

the percentage of non-protein nitrogen. Blish also determined the amount of amino, ammonia, and amide nitrogen in the non-protein nitrogen. He found the percentage of ammonia and amide nitrogen remained about constant for wheat frosted at various stages of maturity, while the percentage of amino nitrogen decreased with the maturity of the sample. These investigations have raised the question as to whether or not the high amino nitrogen content of wheats frosted at various stages of maturity is due to the freezing or to the inhibition of the development of the wheat kernel at earlier stages of growth.

Experimental

The amino-nitrogen content of the normal developing wheat kernel was first studied. According to Fisher, as the amino acids are built up into protein the amino groups disappear, forming the peptide linkages of the protein. Therefore a study of the amino nitrogen content of the kernel should afford an indirect method of following protein synthesis.

Amino-nitrogen in the normal wheat kernel.—Enough kernels to make at least 6 grams of fresh material were picked out by hand, were thoroly mixed and 2 gram portions taken (1) for the determination of amino nitrogen in the fresh kernels, (2) for air drying and subsequent determination of amino-nitrogen in the air-dried material, and (3) for moisture and protein determinations. It is probable that the moisture content of the most immature kernels is too low, for it was almost impossible, owing to the time involved, to pick out the kernels without some loss of moisture. As soon as the kernels were picked from a head they were placed in a stoppered bottle. The aliquots for air drying were placed on watch glasses and dried at room temperature, which ranged from 22° to 27°C. The moisture was determined by drying in a vacuum oven. In the most immature samples the weight per kernel was calculated from a count of one 2-gram portion and in the other cases from a count of each of the three portions. Each 2-gram portion in the later stages usually contained within one or two of the same number of kernels.

The amino nitrogen present in the grain was determined as follows: As soon as possible after threshing and weighing, 2 grams of the fresh green kernels was thrown into a small beaker containing 25 cc. of boiling distilled water and boiled about 20 minutes. The kernels and water were then poured into a mortar, ground until the kernels were entirely disintegrated, returned to the beaker together with the washings, and the boiling was continued for

about 20 minutes longer, by which time the volume was reduced to about 15 cc. The mixture and washings were transferred to a 25-cc. volumetric flask, 1.25 cc. of a 20 per cent solution of sodium tungstate was added, the flask was shaken thoroly, four drops of concentrated sulphuric acid were added, the solution was diluted to the mark, and shaken. The precipitated material was thrown out by means of a centrifuge and the amino nitrogen content of 2.00 cc. aliquots determined by means of Van Slyke's (1915) micro apparatus for the determination of free amino groups. This procedure for precipitating the protein gives higher results for the amino nitrogen content and a more turbid filtrate than the copper hydroxide method described by Blish (1918) when applied to this material. The tungstate method used by Folin and Wu (1919) for the precipitation of blood proteins was first applied to flour by Rumsey (1922) who used it in the clarification of flour extracts for sugar determinations.

The procedure designed for the determination of amino nitrogen in the fresh kernels was modified only slightly for the dried kernels. The dried material ground fine was thrown into boiling water, thus eliminating the necessity for grinding in the mortar.

The amino nitrogen content of a spring wheat, Marquis, and a winter wheat, Kanred, was investigated in the summer of 1922. These wheats were both grown under dry-farming conditions by a farmer living near the Montana Agricultural Experiment Station.

At the time this investigation was started the kernel development of the winter wheat had progressed to a considerable extent but kernel formation had just begun in the spring wheat. A description of the condition of the kernels of spring wheat at the time the samples were taken follows:

S-100. Just past the bloom stages, the formation of some kernels not yet begun.

S-101 and S-102. Kernels about half formed.

S-103. Kernels about three-fourths formed.

S-104. Kernels about three-fourths formed, some milk, but mostly a clear liquid.

S-105. Kernels formed and in the milk and clear liquid stage.

S-107. Milk with a small amount of thin dough.

S-109 and S-111. Almost all dough.

S-113. All thin dough.

S-115. Dough beginning to thicken. Field as a whole beginning to turn yellow.

S-117. Thick dough.

S-119. Thick dough. Farmer began harvest of main part of the field.

S-121. Thick dough.

S-123. Could still cut kernels with fingernail.

S-125. Some kernels could not be cut with fingernail but most of them could be cut with difficulty.

S-127. Kernels could not be cut with fingernail. Kernels were becoming translucent.

S-129. Kernels hard and translucent.

In order to show the development of the kernel of this series in terms of weight, the wet and vacuum-dry weights per kernel taken from columns 5 and 6 of Table I are plotted against age (see Fig. 4). It is interesting to note that the farmer chose to cut his wheat at a time when the kernel contained nearly the maximum quantity of moisture altho, of course, not its highest percentage amount. The dry weight per kernel had not reached the maximum but the kernel would undoubtedly increase in dry weight by further translocation while in the shock.

The results obtained with the wheat from these two fields are presented in Tables I and II. The amount of amino nitrogen obtained in each case has been multiplied by the factor 5.7 in order to show the amount of protein which presumably could be synthesized from it and the results of this calculation have been recorded in the tables under the headings "Amino nitrogen expressed as protein ($\text{NH}_2\text{-NX}5.7$)."

As an example of this calculation we may take the sample S-100, column 10, Table I, which contained 0.463 mgms. of amino nitrogen per gram of dry matter. This amino nitrogen if synthesized to protein would presumably yield 0.463×5.7 , or 2.64 mgms. of protein, which would correspond to 2.64 per cent of the original wheat as protein. It should be remembered in this connection that only half of the nitrogen of asparagine is liberated in the Van Slyke apparatus and consequently a larger percentage of the total nitrogen may be present in simple form than is indicated by the amino nitrogen determinations.

In order to show the relationship between the amino nitrogen and moisture content of the developing kernel, the results were plotted in Figure 1, using the moisture content as abscissa and the amino nitrogen, expressed as protein on the wet basis, as ordinates. The results of a similar study which are presented in Table VIII are also shown in Figure 1.

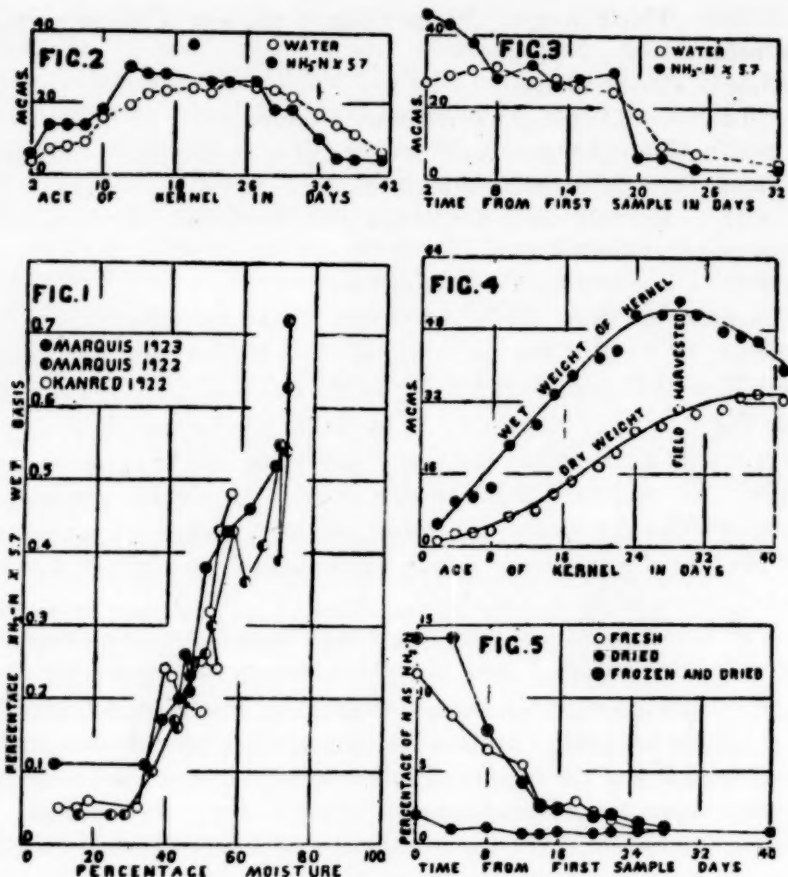


Fig. 1. Relation of the amino nitrogen expressed as protein in the developing wheat kernel to the moisture content of the kernel, the fresh material being analyzed at various stages of growth.

Fig. 2. Actual water content per kernel, in milligrams, for the 1922 samples of Marquis wheat at various stages of development and also milligrams of amino nitrogen, expressed as protein, multiplied by 200 to make the curve approximately correspond with the curve for the water content per kernel.

Fig. 3. The same relationship as Figure 2 but for the 1922 samples of Kanred wheat.

Fig. 4. Change in wet and dry weight per kernel of the 1922 samples of Marquis wheat as the kernel develops.

Fig. 5. Percentage of total nitrogen present in amino form at various stages of development of 1923 samples of Marquis wheat, (1) in the fresh kernels, (2) in kernels threshed from heads which were harvested and air dried before threshing and (3) in kernels threshed from heads which were frozen immediately after gathering and then air dried before threshing.

An examination of Figure 1 indicates that the points fall very nearly on a straight line for the middle period of kernel development. The experimental error is rather large and from the data

it is impossible to tell exactly whether this portion of the curve is actually straight or slightly curved; but if this portion is curved, the curve is so slight that for the purpose of this discussion it will be considered straight.

Thus Figure 1 indicates that for a middle period in the development of the kernel there is a rather direct relationship between moisture and amino nitrogen. Apparently this middle period is limited at the beginning of kernel development by a period when the amino nitrogen content is not related to the moisture content in this simple manner; and at the end, after the moisture in the kernel has a decrease below a limiting value, where there is then no further decrease of amino nitrogen with a decrease in moisture. That the wheats may not all develop to the same amino nitrogen content is also shown by the difference in the points where the line begins to go parallel with the abscissa.

The relationship of amino nitrogen to moisture is shown graphically in another way. The data for Figure 2 are taken from Table I, columns 7 and 8 and those for Figure 3 from Table II, columns 7 and 8. In these figures the abscissa represent the time of sampling; the ordinates, the calculated water content per kernel expressed in milligrams for one curve and the amino nitrogen expressed as protein per kernel multiplied by 200 to make the values approximately correspond in magnitude for graphical comparison, in the other. It is seen that the curves for water and amino nitrogen correspond.

The data thus far discussed indicate an equilibrium for the middle period of kernel development between the amino and the more complex nitrogen compounds that is governed by the moisture content. Thus we would expect that a decrease in amino nitrogen would take place on air drying the threshed kernels.

It was found that the amino nitrogen content of threshed immature wheat kernels, after air drying in watch glasses in the laboratory, was much less than at the time when they were threshed out. This is shown by a comparison of columns 10 and 12 of Tables I and II and indicates protein synthesis due to desiccation. This result is apparently in agreement with the findings of Eckerson (1917) and Olson (1917), who emphasize the importance of desiccation in protein formation in the wheat kernel.

TABLE I
 SPRING WHEAT, MARQUIS, 1922 CROP, THRESHED
 Amino nitrogen content at different stages of growth of the fresh and the air dried, isolated kernels, together with their wet and dry weight.

Lab. No.	Date	Age of kernel days	Moisture %	Weight per kernel		Water content per kernel mgms.	Amino nitrogen in kernels expressed in protein (NH ₂ -N×5.7)				Total protein (N×5.7), dry basis	
				Wet mgms.	Dry mgms.		Per kernel Mgms.	Fresh kernels Wet basis %	Dry basis %	Fraction of total protein %		Air-dried kernels, dry basis %
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	July											
S-100	27	2	72.6	5.2	1.4	3.8	0.03	0.72	2.64	2.49
S-101	29	4	72.8	9.9	2.7	7.2	0.07	0.72	2.63	1.87	13.43
S-102	31	6	72.6	10.9	3.0	7.9	0.07	0.63	2.31	16.9	2.13	13.65
August												
S-103	2	8	72.5	12.8	3.5	9.3	0.07	0.54	1.96	15.1	1.58	13.00
S-104	4	10	70.9	22.5	6.5	16.0	0.09	0.39	1.36	10.8	0.83	12.63
S-105	7	13	70.7	27.1	7.9	19.2	0.15	0.55	1.88	14.1	0.88	13.36
S-107	9	15	66.1	33.9	11.5	22.4	0.14	0.41	1.21	10.2	0.43	11.90
S-109	11	17	61.5	37.8	14.5	23.3	0.14	0.36	0.94	8.1	0.43	11.66
S-111	14	20	57.7	41.7	17.7	24.0	0.18	0.43	1.01	8.5	0.31	11.90
S-113	16	22	52.4	43.5	20.7	22.8	0.13	0.30	0.63	5.9	0.23	10.71
S-115	18	24	50.9	51.3	25.2	26.1	0.13	0.26	0.52	4.7	0.10	11.05
S-117	21	27	47.5	51.3	26.9	24.4	0.13	0.25	0.47	3.9	0.13	12.10
S-119	23	29	43.3	54.1	30.6	23.5	0.09	0.16	0.29	2.3	0.15	12.88
S-121	25	31	42.5	51.3	29.5	21.8	0.09	0.17	0.29	2.1	0.09	14.15
S-123	28	34	36.1	47.6	30.5	17.1	0.05	0.10	0.15	1.2	0.05	12.38
S-125	30	36	28.9	46.5	33.1	13.4	0.02	0.04	0.06	0.5	0.05	12.84
Sept.												
S-127	1	38	25.2	45.5	34.0	11.5	0.02	0.04	0.06	0.5	0.04	11.98
S-129	4	41	16.3	39.2	32.6	6.6	0.02	0.04	0.05	0.4	0.02	11.80

* Main part of field harvested.

TABLE II

WINTER WHEAT, KANRED, 1922 CROP

Amino nitrogen content at different stages of growth of the fresh and the air dried isolated kernels, together with their wet and dry weight.

Lab. No.	Date	Time from first sample days	Moisture %	Weight per kernel		Water content per kernel	Amino nitrogen in kernels expressed in protein (NH ₂ -N × 5.7)						Total protein (NX5.7), dry basis
				Wet	Dry		Fresh kernels			Fraction of total protein	Air-dried kernels, dry basis		
							Per kernel	Wet basis	Dry basis		%	%	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	
	July			mgms.	mgms.	mgms.	mgms.	%	%	%	%	%	
S-3	29	0	57.0	47.2	20.3	26.9	0.23	0.48	1.12	10.4	0.60	10.80	
S-5	31	2	54.2	53.4	24.5	28.9	0.22	0.43	0.90	8.7	0.39	10.40	
	August												
S-7	2	4	51.9	58.0	27.9	30.1	0.19	0.32	0.66	5.8	0.21	11.46	
S-9	4	6	54.0	58.8	27.1	31.7	0.14	0.24	0.53	4.5	0.18	11.82	
S-11	7	9	49.6	59.7	32.1	27.6	0.16	0.25	0.49	4.4	0.13	11.02	
S-13	9	11	43.8	64.5	36.3	28.2	0.13	0.20	0.35	2.9	0.10	11.92	
S-15	11	13	41.3	61.6	36.1	25.5	0.14	0.23	0.39	3.4	0.11	11.33	
S-17	14	16	39.7	61.6	37.1	24.5	0.15	0.24	0.39	3.3	0.14	11.65	
S-19	16	18	32.2	58.0	39.3	18.7	0.03	0.05	0.07	0.6	0.05	11.03	
S-21	18	20	18.7	50.0	40.6	9.4	0.03	0.06	0.08	0.7	0.08	11.13	
S-23	21	23	15.4	48.7	41.3	7.4	0.02	0.05	0.06	0.5	0.06	11.69	
S-25	28	30	10.3	41.8	37.4	4.4	0.02	0.05	0.06	0.5	0.06	11.43	

* Main part of field harvested.

This conclusion as to the importance of desiccation has been reached by observing the final result, that is, a disappearance of the simpler nitrogen compounds or an increase in protein material, and attributing this change to a decrease in moisture. Two variable factors, however, have been operating in the experiments on the effect of desiccation previously performed (1) time and (2) a decrease in moisture content. Thus the decrease in simpler nitrogen compounds may be due to either desiccation or time or both.

A considerable amount of immature wheat was picked out and divided into 2-gram aliquots which were kept under various conditions and then weighed and the amino nitrogen determined. The experiment was run in duplicate; the results will be found in Table III, which is largely self-explanatory. The moisture content was calculated using the original dry matter content at the start.

Table III shows that when threshed immature wheat kernels were kept in an atmosphere saturated with water vapor, thus preventing desiccation and making it possible to observe the effect of the time factor alone, a marked decrease in amino nitrogen took place. When the kernels were dried rapidly, thus keeping the time factor approximately constant while varying the moisture content, a decrease in amino nitrogen did not take place.

The objection may be raised to these two experiments that they were carried out at 100° and 40°C., thus introducing a third variable, temperature. If we compare those experiments in which the wheat was kept for 48 hours at room temperature, we find that the amino nitrogen decreased to its lowest value in the samples where no desiccation occurred. The kernels kept over concentrated sulphuric acid and those dried in a current of rapidly moving air produced by an electric fan showed a decrease in amino nitrogen from the original of 43 and 38 per cent, respectively, while kernels kept in a saturated atmosphere showed a decrease of 70 per cent. The experiments recorded in Tables IV and V, which will be discussed in detail later, show that the amino nitrogen decreases slowly when there is no desiccation. The kernels kept over sulphuric acid decreased in moisture from 65 to 25.6 per cent in 48 hours, while those dried before an electric fan decreased from 65 to 30.5 per cent in the same time. It is thus seen that desiccation carried out in this way required a period of time sufficiently long to permit the disappearance of some of the amino nitrogen. If desiccation also tended to produce a decrease in amino nitrogen, these two experiments should show less amino nitrogen than the ones in which no desiccation occurred. The reverse is actually the case.

It should be borne in mind that other steps in the synthesis of protein may not be affected by these factors in the same way as the amino nitrogen but so far as a decrease in amino nitrogen indicates protein synthesis, desiccation has very little to do with the process other than to stop it when a limiting moisture content has been reached below which insufficient moisture is present for the synthetic reaction to take place.

TABLE III

RELATION OF METHOD OF KEEPING TO THE AMINO NITROGEN CONTENT EXPRESSED AS PROTEIN (AMINO N \times 5.7) OF THRESHED, IMMATURE WHEAT KERNELS

The moisture content at the start was 65.0 per cent, that at the time of analysis is given in the third column.

Method of treatment	NH ₂ -N as protein dry basis	Change in NH ₂ -N from start	Moisture content
	%	%	%
At start	1.26		65.0
	1.32	0	65.0
Vacuum dried for 15 hours at 100°C.....	1.23		0.0
	1.24	-4	0.0
Kept at room temperature in stoppered test tube 48 hours	0.38		64.3
	0.39	-70	64.3
Kept at room temperature in stoppered test tube 72 hours	0.26		63.9
	0.23	-81	63.2
Dried in vacuo over H ₂ SO ₄ at 40°C. for 3 hours	1.33		6.4
	1.31	+2	6.8
Dried at room temperature over H ₂ SO ₄ for 48 hrs.	0.74		25.7
	0.74	-43	25.6
Air dried at room temperature in front of electric fan for 48 hours	0.81		30.9
	0.79	-38	30.1
	0.54		10.8
Air dried in laboratory at room temperature.....	0.55	-58	10.0

Table III also indicates the importance of the method of drying plant material that is later to be subjected to analysis for nitrogen distribution. Apparently all previous analyses of the nitrogen distribution in the developing kernel have been carried out on air-dried material and can therefore hardly be said to represent conditions as they exist in the growing plant. Chibnall (1922) and Link and Schulz (1924) have recently called attention to the effect of drying plant tissue on the nitrogen distribution. Their papers give a review of the literature.

The effect of freezing on the amino nitrogen of the growing kernel was studied in three different ways, (1) on the threshed immature kernel, (2) on heads plucked at various stages of growth, part of which were frozen, and (3) freezing of wheat growing in pots.

TABLE IV

CHANGE IN AMINO NITROGEN ON STORING OF THRESHED FROZEN AND NON-FROZEN KERNELS IN STOPPERED TEST TUBES FOR DIFFERENT PERIODS OF TIME

The original moisture content was 46.5 per cent. The amount of amino nitrogen obtained was multiplied by 5.7 to change it to the amount of protein which presumably could be synthesized from it. The percentage of this calculated protein equivalent present in the dry matter of the kernel is given in columns 2, 4, 6, 8. The percentage change in amino nitrogen calculated on the basis of the original value at the start as having 0 per cent change, is given in columns 3, 5, 7, and 9.

Time, hours	Temperature 25°C					Temperature 35°C				
	Non-frozen			Frozen		Non-frozen			Frozen	
	NH ₂ -N × 5.7 in wheat		Decrease	NH ₂ -N × 5.7 in wheat		NH ₂ -N × 5.7 in wheat		Decrease	NH ₂ -N × 5.7 in wheat	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
0	%	%	%	%	%	%	%	%	%	%
6	0.75	0	0.73	0	0.75	0	0.73	0	0.73	0
12	0.71	5	0.77	5	0.70	7	0.89	22	0.89	22
18	0.45	..	0.90	23	0.98	34	0.98	34
24	0.31	40	0.50	33
30	0.31	59	0.87	19	0.36	52	1.03	41	1.03	41
36	0.20
42	0.20	73	0.94	29	0.22	71	1.05	44	1.05	44
48	0.16	..	0.83	14
54	0.10	79	0.23	67	0.99	36	0.99	36
66	0.10	87	0.10	87
96	0.83*	14	1.03*	41	1.03*	41

*Sample showed evidence of the growth of micro-organisms.

Effect of freezing on threshed kernels.—In order to study the effect of freezing on the amino nitrogen, a sufficient amount of wheat was picked out by hand and divided into 2-gram aliquots which were placed in test tubes and stoppered. Half of the samples were frozen. The frozen and non-frozen samples were then subjected to the same conditions of storage and were analyzed at intervals for amino nitrogen. The freezing was accomplished by placing the test tubes in the hardening room of an ice cream manufacturing plant for at least over night. The temperatures ranged from -20° to -28°C . Wheat grains from several different fields of both spring and winter wheat were studied, the result being always essentially the same. A typical example of the results obtained is given in Table IV, which also shows the effect of storage at different temperatures. The amino nitrogen in the non-frozen samples steadily decreased while that in the frozen samples steadily increased; at least up to a point at which the growth of micro-organisms was apparent.

There seemed to be little difference in the rate of disappearance of amino nitrogen from the non-frozen kernels stored at 25° and 35°C . At 10°C ., however, the rate is decreased, as shown in Table V. The amino nitrogen increased more rapidly in the frozen kernels stored at 35°C . than in those stored at 25°C .

In three instances out of the fourteen that the experiment was performed, the frozen kernels failed to show an increase in amino nitrogen but tended to remain constant or decrease only slightly. In these three cases, however, the difference between the behavior of the amino nitrogen in the frozen and the non-frozen kernels was so marked that the effect of the freezing could easily be recognized. An example of this behavior is given in Table V, which also shows the effect of storing at two different temperatures. The amino nitrogen did not disappear as rapidly from the non-frozen wheat stored at 10°C . as it did from that stored at 35°C . The tendency of high temperature apparently is to increase the rate of disappearance of amino nitrogen in the non-frozen wheat and to increase the rate of appearance in the frozen material.

TABLE V

CHANGE IN AMINO NITROGEN ON STORING OF THRESHED FROZEN AND NON-FROZEN KERNELS IN STOPPERED TEST TUBES FOR DIFFERENT PERIODS OF TIME

The original moisture content was 50.6 per cent. The amount of amino nitrogen obtained was multiplied by 5.7 to change it to the amount of protein which presumably could be synthesized from it. The percentage of this calculated protein equivalent present in the dry matter of the kernel is given in columns 2, 4, 6, and 8. The percentage change in amino nitrogen calculated on the basis of the original value at the start as having 0 per cent change, is given in columns 3, 5, 7, and 9.

Time, hours	Temperature 10°C				Temperature 35°C			
	Non-frozen		Frozen		Non-frozen		Frozen	
	NH ₂ -N × 5.7 in wheat	Decrease	NH ₂ -N × 5.7 in wheat	Decrease	NH ₂ -N × 5.7 in wheat	Decrease	NH ₂ -N × 5.7 in wheat	Decrease
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
0	% 0.77	% 0	% 0.77	% 0	% 0.77	% 0	% 0.77	% 0
15	0.54	30	0.43	44
24	0.43	44	0.43	44	0.50	35	0.60	22
33	0.41	47	0.60	22
39	0.30	61	0.20	74
48	0.20	74	0.47	39	0.03	96	0.63	18
65	0.20	74	0.51	34	0.03	96	0.63	18
72	0.20	74	0.47	39	0.06*	92	0.54*	30

*Sample showed evidence of the growth of micro-organisms.

TABLE VI

AMINO NITROGEN EXPRESSED AS PROTEIN ($\text{NH}_2\text{-N} \times 5.7$) IN FRESHLY THRESHED KERNELS HARVESTED AT VARIOUS STAGES OF DEVELOPMENT AND IN FROZEN AND NON-FROZEN THRESHED KERNELS WHICH HAD STOOD 48 HOURS AT ROOM TEMPERATURE IN AN ATMOSPHERE SATURATED WITH WATER VAPOR

Also amino nitrogen expressed as protein in kernels threshed from heads harvested at the same stages of growth but which had previously been either air dried after harvesting or frozen and then air dried. Results are expressed on the dry basis.

Lab.	No.	Time from start days	Moisture content %	At start %	Amino Nitrogen $\times 5.7$ in dry matter					
					In kernels after 48 hrs. in saturated atmosphere			In kernels threshed from heads after air drying		
					Non-frozen		Frozen	Non-frozen		Frozen
					In dry matter	Decrease from start	In dry matter	In dry matter	Decrease from start	In dry matter
					%	%	%	%	%	%
					2.38	0.31	82	2.04
S-131	0		69.4	1.71	+40	0.12	84	+19
S-137	12		50.6	0.77	0.10	87	-38	0.11	74	-18
S-139	14		46.5	0.42	0.16	62	+40	0
S-143	18		45.0	0.48	0.09	81	+23	0.12	67
S-145	20		43.5	0.36	0.15	58	+41	0.14	48	-8
S-147	22		38.7	0.27	0.19	60	+57	0.34	+26

In order to gain some idea as to the influence of the stage of development of the kernel on the change in amino nitrogen due to freezing, kernels were taken from a field at intervals, and analyzed for amino nitrogen, (1) at once, (2) after standing 48 hours at room temperature in an atmosphere saturated with water vapor, and (3) after freezing and standing 48 hours at room temperature in an atmosphere saturated with water vapor. At these same stages of development a large sample of heads was collected. Half of this sample was at once placed in a room to air dry and the other half was frozen before being air dried. After air drying the wheat was threshed and its amino nitrogen content determined. The results obtained are given in Table VI and are expressed in terms of the percentage of amino nitrogen expressed as protein ($\text{NH}_2\text{-N} \times 5.7$) in the total dry matter. The results for each stage of development are also expressed as percentage change from the amino nitrogen content of the fresh kernels at the start, that is, at the time of harvesting.

Table VI shows that with one exception the threshed kernels which were frozen and then kept at room temperature in an atmosphere saturated with water vapor for 48 hours before analysis, all contained more amino nitrogen than did the freshly harvested kernels, the percentage increase being about the same. The non-frozen kernels which were so treated all showed a decrease, the percentage decrease being rather irregular. The results obtained from the kernels threshed from the frozen heads showed only a slight average increase while those from the non-frozen heads showed a marked decrease in every instance, the percentage decrease growing progressively less as the kernel developed. Non-frozen kernels with an original moisture content of 50 per cent or less all seemed to decrease to approximately the same amino nitrogen content irrespective of the original value. As the kernel develops the amino nitrogen content decreases and so does the amino nitrogen of the frozen kernels. It thus seems that the amino nitrogen content of the frozen kernels is related to the amino nitrogen content of the kernels at the time of freezing.

As a further indication that protein synthesis takes place in the threshed kernel on standing and of the effect of freezing on this change, the following experiment was performed. Wheat was collected at various periods of growth, the kernels were picked out and divided into 10-gram aliquots. Two such aliquots were at once separately mascerated in a mortar with 5 per cent potassium sulphate and made up to a final volume of 100 cc. The mixture was

then shaken for 2 hours in a mechanical shaker, the material centrifuged, and the protein determined in 50 cc. of the supernatant liquid. Two aliquot 10-gram portions were kept 48 hours in stoppered bottles and then analyzed, and 2 others were frozen and kept in stoppered bottles for 48 hours at room temperature before analysis. The most nitrogen extractable with potassium sulphate was found in the fresh kernels. After the non-frozen kernels had stood for 48 hours the amount of nitrogen extractable with potassium sulphate had decreased to a considerable extent, while with the kernels which were frozen and then allowed to stand for 48 hours the decrease was not so marked but apparently approached the decrease of the non-frozen kernels in the later stages of development. The results obtained are given in Table VII. Experiments have been conducted to show whether this decrease in potassium sulphate-soluble protein is accompanied by an increase in the gliadin or glutenin fraction or both, but owing to the difficulty of quantitatively determining these fractions no conclusion can as yet be drawn.

TABLE VII

PROTEIN ($N \times 5.7$) EXTRACTED FROM MASSERATED WHEAT KERNELS BY 5 PER CENT POTASSIUM SULPHATE

Results expressed on the dry basis.

Lab. No.	Fresh kernels		Stood 48 hours before analysis		Frozen and stood 48 hours before analysis	
	Total dry matter	Total Nitrogen	Total dry matter	Total nitrogen	Total dry matter	Total nitrogen
	%	%	%	%	%	%
S-135	5.15	33.8	3.80	25.0
S-135	4.98	32.7	3.28	21.5
S-137	4.24	29.9	2.88	20.3	3.64	25.7
S-137	3.75	26.5	2.94	20.7	3.68	26.0
S-139	3.91	24.3	2.81	17.5	3.55	22.0
S-139	3.57	22.1	2.68	16.6	3.63	22.5
S-143	4.24	25.9	3.07	18.7	3.03	18.5
S-143	4.11	25.1	3.38	20.6	3.33	20.4
S-145	4.00	23.9	3.08	18.4	3.42	20.4
S-145	4.00	23.9	2.96	17.7	3.42	20.4
S-147	3.56	21.5	2.95	17.8	2.99	18.1
S-147	3.54	21.3	2.80	16.9	2.82	17.0

During the course of these investigations some observations were made on the color change of the threshed kernels from green to yellow. The change seems to be independent of sunlight but is accelerated by higher temperatures. The kernel must contain a certain amount of water for this change to take place. If the kernels are dried fairly rapidly they may be dried even at room temperature before the color change takes place. Freezing either prevents or retards this color change to such an extent in the more immature kernels that it does not take place, but in the later stages of development freezing only decreases the rate.

Effect of freezing on heads plucked at various stages of development.—A portion of a 1923 crop of Marquis spring wheat was purchased from a farmer living near the Montana Agricultural Experiment Station. This wheat was grown under a system of dry farming. At two- to four-day intervals during the development of the kernel, portions of the wheat were harvested. In order to restrict the amount of material flowing into the kernel after harvest, only the heads were gathered. The gathering of bulk samples of the heads was facilitated by the use of an improvised comb made by driving headless nails into a short piece of wood. Enough heads were secured at a time to fill six 24-pound flour sacks. Three of these sacks were placed in the hardening room of an ice cream manufacturing plant for 48 hours, after which they were spread on the floor of a large room to dry. The other three sacks picked at the same time were at once spread on the floor to dry. At the time each sample was collected, the amino nitrogen and moisture content of the fresh kernels were determined. After the heads had become dry they were threshed and the wheat was milled. The results of the amino nitrogen determinations expressed as protein ($\text{NH}_2\text{-N} \times 5.7$) are given in Table VIII. This table also gives the percentage of the total nitrogen which was present in the amino form, the data for the total protein given in Table IX being used in this calculation.

One of the most interesting points brought out in Table VIII and which confirms the findings with the threshed kernels, is that the wheat from the frozen heads showed a tendency to contain as much amino nitrogen as did the wheat at the time of freezing or more; while the non-frozen samples contained much less nitrogen in the amino form than did the fresh kernels at the time of picking. Beginning with sample S-137 which contained approximately 50 per cent moisture. The dried wheat samples threshed from the non-frozen heads all contained approximately the same amount of amino nitrogen. The results obtained for the amino nitrogen expressed as protein for the fresh kernels and for the kernels threshed from the frozen and non-frozen dried heads are expressed graphically in Figure 5. There is a positive correlation of the amino nitrogen content of the wheat with that of the flour. The flour apparently does not have quite so high a percentage of its nitrogen in amino form as does the wheat from which it was milled.

TABLE VIII

AMINO NITROGEN EXPRESSED AS PROTEIN ($\text{NH}_2\text{-N} \times 5.7$) IN FRESH KERNELS PICKED AT VARIOUS STAGES OF GROWTH, AND ON WHEAT THRESHED, AFTER AIR DRYING, FROM FROZEN AND NON-FROZEN HEADS, TOGETHER WITH THE AMINO NITROGEN FRACTION PRESENT IN THE FLOUR MILLED FROM THESE SAMPLES

Lab. No.	Date	Time from first sample days	Weight per 1,000 kernels dried in the head		Amino nitrogen expressed as protein ($\text{NH}_2\text{-N} \times 5.7$)										Percentage of total nitrogen present as amino nitrogen					
			Dry basis		Fresh kernels		Wheat from dried heads, dry basis		Flour from wheat dried in heads, dry basis		Fresh kernels		Non-frozen		Wheat dried in heads		In flour from wheat dried in heads			
			grams	%	Wet basis	Dry basis	%	%	%	%	%	%	Frozen	Non-frozen	%	%	Frozen	Non-frozen	Frozen	Non-frozen
S-131	Aug. 9	0	9.15	69.4	0.52	1.71	0.31	2.04	0.34	1.21	11.7	1.9	14.0	2.3	8.3					
S-133	13	4	13.88	62.5	0.46	1.23	0.15	1.97	0.14	1.22	8.8	1.0	13.6	0.9	8.4					
S-135	17	8	20.57	56.2	0.43	0.98	0.17	1.18	0.04	0.80	6.4	1.1	7.8	0.3	5.2					
S-137	21	12	24.50	50.6	0.38	0.77	0.12	0.63	0.04	0.46	5.4	0.8	4.2	0.3	3.2					
S-139	23	14	25.67	46.5	0.23	0.42	0.11	0.42	0.07	0.30	2.5	0.7	2.6	0.4	1.9					
S-141	25	16	27.53	46.5	0.21	0.40	0.13	0.42	0.09	0.31	2.5	0.8	2.4	0.6	1.8					
S-143	27	18	28.50	45.0	0.26	0.48	2.9					
*S-145	29	20	29.07	43.5	0.20	0.36	0.12	0.33	0.09	0.22	2.2	0.7	1.9	0.6	1.4					
S-147	31	22	30.85	38.7	0.17	0.27	0.14	0.34	0.07	0.32	1.6	0.8	2.0	0.4	2.0					
	Sept.																			
S-149	3	25	32.66	34.1	0.11	0.17	0.14	0.24	0.08	0.13	1.0	0.8	1.4	0.5	0.8					
S-151	6	28	32.07	0.15	0.21	0.07	0.10	0.8	1.2	0.4	0.6					
S-153	18	40	9.3	0.11	0.12	0.12	0.04	0.7	0.7	0.3					

*Main part of field harvested.

Effect of freezing on Marquis wheat grown in pots in the greenhouse.—Plantings were made on November 1 and December 1, 1922. The growth of the wheat was very slow. Both plantings headed at very nearly the same time and on May 27, 1923, were in the dough stage. On this date some of the pots containing the growing wheat were placed in the hardening room of an ice cream manufacturing plant where the temperature ranged around -20°C . Pots containing the November 1 planting were subjected to the following different lengths of time of freezing, $1\frac{1}{4}$, $2\frac{1}{4}$, and 14 hours; pots from the December 1 planting were subjected to the 14-hour period only. At the end of the freezing period the pots were returned to the greenhouse and the change in the amino nitrogen content of the kernels was compared with that in pots of wheat which had not been frozen. Four pots of growing wheat received each treatment.

TABLE IX

TOTAL PROTEIN ($\text{N} \times 5.7$), ON THE DRY BASIS, IN THE FRESH WHEAT, IN WHEAT DRIED IN THE HEAD, IN WHEAT FROZEN AND DRIED IN THE HEAD, AND IN FLOUR FROM THE NON-FROZEN AND FROZEN WHEAT DRIED IN THE HEAD

Lab. No.	Fresh kernels	Wheat		Flour	
		Non-frozen	Frozen	Non-Frozen	Frozen
	%	%	%	%	%
S-131	14.60	16.13	14.59	15.07	14.60
S-133	14.03	15.02	14.49	14.39	14.47
S-135	15.21	15.40	15.19	14.62	15.27
S-137	14.18	15.77	15.10	15.10	14.47
S-139	16.12	16.19	16.18	15.62	15.47
S-141	15.85	17.00	17.29	15.91	17.14
S-143	16.37
S-145	16.72	16.73	17.08	16.08	16.37
S-147	16.56	17.08	16.70	16.39	16.07
S-149	16.80	16.98	17.06	16.42	16.09
S-151	16.38	17.81	17.03	16.47	16.02
S-153	16.65	15.82

The frozen plants began to dry after being returned to the greenhouse. At the time of each determination of amino nitrogen, an aliquot of the threshed kernels was allowed to air dry and the amino nitrogen was determined after the kernels were air dry. The results obtained are given in Table X.

It was very difficult to sample the small amount of wheat which could be grown in four 2-gallon pots. The results in Table X, therefore, are rather erratic, but taking the table as a whole it shows that the results obtained by freezing the growing plant confirm the previous findings—that under normal conditions the amino nitrogen tends to decrease either in the growing plant or in the isolated kernel, while with frozen wheat the general tendency is for the amino nitrogen to remain about the same or to increase.

TABLE X

AMINO NITROGEN EXPRESSED AS PROTEIN ($N \times 5.7$) OF WHEAT KERNELS FROM PLANTS FROZEN WHILE GROWING IN POTS AND ANALYZED AFTER VARIOUS PERIODS OF TIME; ALSO OF KERNELS THRESHED AT THESE VARIOUS TIMES AND AIR-DRIED BEFORE ANALYSIS

All results are expressed on the dry basis.

PLANTING OF NOVEMBER 1

Time after freezing	Controls not frozen		1½ hours freezing		2¼ hours freezing		14 hours freezing	
	Green	Air-dried	Green	Air-dried	Green	Air-dried	Green	Air-dried
Days	%	%	%	%	%	%	%	%
1	0.25	0.14	0.29	0.24	0.29	0.31	0.27	0.25
3	0.32	0.26	0.36	0.35	0.34	0.30	0.38	0.38
5	0.26	0.13	0.29	0.26	0.37	0.34	0.36	0.35
7	0.36	0.33	0.37	0.35	0.47
12	0.40	0.38	0.25	0.37	0.61
14	0.14	0.07
Moisture								
1	38.1	38.1	32.4	34.2	34.2	34.2	34.2	38.7
3	42.4	42.4	27.0	25.6	25.6	25.6	25.6	30.2
5	35.9	35.9	22.8	23.6	23.6	23.6	23.6	24.3
7	18.9	18.1	18.1	18.1	18.1	18.7
12	16.6	16.3	16.3	16.3	16.3	18.8
14	21.0	21.0
PLANTING OF DECEMBER 1								
1	0.34	0.21	0.35	0.42
3	0.37	0.27	0.51	0.53
5	0.35	0.20	0.50	0.57
7	0.55	0.63
12	0.63
14	0.28	0.15
22	0.14
Moisture								
1	47.8	47.8	43.7	43.7
3	48.1	48.1	34.6	34.6
5	46.0	46.0	29.3	29.3
7	22.8	22.8
12	19.4	19.4
14	34.0	34.0
22	18.8	18.8

Comparison of different protein precipitants.—All of the amino nitrogen determinations reported thus far were carried out on the supernatant liquid after precipitating with tungstic acid as previously described. In order to see how this method of precipitation compared with other methods, a series of precipitating agents was tried on the same sample of immature wheat.

Four grams of freshly threshed kernels was thrown into a small beaker containing about 25 cc. of boiling distilled water, and boiled approximately 10 minutes to stop all enzyme action. The kernels and liquid were then decanted into a mortar and ground to a paste. This paste, together with the washings, was returned to the beaker and boiled for 30 minutes. The precipitating agents are given in Table XI, which is self-explanatory. The solution in each case was made up to a final volume of 100 cc. The precipitate was thrown down in a centrifuge bottle and 25 cc. aliquots of the supernatant liquid were evaporated on a hot plate to a volume of 1 cc. This concentrated liquid was transferred to a micro Van Slyke amino nitrogen apparatus and the beaker washed twice with 1 cc. portions of distilled water. The results are given in Table XI.

TABLE XI

AMINO NITROGEN IN THE FILTRATE AFTER PRECIPITATING THE PROTEIN FROM IMMATURE WHEAT BY VARIOUS METHODS

	Nitrogen gas after subtracting blank cc.	NH ₂ N in 1 gram wet weight, mgms.
Sample evaporated to a volume of 15 cc. and made up to 100 cc. with 95% alcohol	1.00	0.48
	0.95	0.45
	0.98	0.47
Sample evaporated to a volume of 35 cc., 7 cc. of a 15% solution of sodium tungstate and 10 drops of concentrated sulphuric added. Made up to 100 cc. with 95% alcohol.....	0.73	0.35
	0.76	0.36
	0.81	0.39
Sample evaporated to a volume of 33 cc., 2 cc. of a 15% solution of sodium tungstate and 4 drops of concentrated sulphuric added. Made up to 100 cc. with 95% alcohol.....	0.94	0.45
	1.02	0.49
	1.01	0.48
Sample evaporated to a volume of 35 cc., 1 cc. of a saturated solution of ZnCl ₂ in alcohol added and made up to a final volume of 100 cc. with 95% alcohol.....	1.08	0.52
	1.07	0.51
Sample evaporated to about 50 cc., 2 cc. of normal CuSO ₄ added, shaken, allowed to stand 5 minutes, then 2 cc. of normal NaOH added, shaken, made to a volume of 100 cc. with water, shaken, and then two drops of normal CuSO ₄ added	0.91	0.43
	0.94	0.45
	0.87	0.42
Duplicate of above.....	0.91	0.43
	0.87	0.42

By the heating process the starch was gelatinized and the clearness of the filtrate and ease of concentration depended largely upon the completeness of the precipitation of the starch. Tungstic acid in aqueous solution was especially poor as a precipitating agent for the gelatinized starch, and it was impossible to concentrate the filtrates to an appreciable extent. This is the reason results obtained with this method are not included in Table XI. The copper precipitation gave clear filtrates which in some cases showed a slight scum on concentration to 1 cc. No difficulty was encountered in concentrating those filtrates if a considerable quantity of alcohol was used in the precipitation. The combination of adding enough sodium tungstate to correspond to about 1 gram, then acidifying, and making up to the volume of 100 cc. with alcohol seemed to give the lowest amino nitrogen content in the filtrate.

In some other work which was under way on the amino nitrogen content of the immature kernel, the amino nitrogen was determined by both the copper hydroxide method described in Table XI and the tungstic acid method used in this paper. In a comparison of 17 cases selected at random, the results obtained for amino nitrogen by the copper hydroxide method averaged 26 per cent lower than those obtained by precipitation with tungstic acid. The general effects of freezing, standing, and the relationship of moisture content of the kernel to its amino nitrogen were apparently the same using either method.

DISCUSSION

The results of the investigation of the change of nitrogen compounds during the development of the wheat kernel and the effect of freezing that are reported here are in the nature of a report of progress and the discussion should be considered more in the light of a possible explanation rather than of definite conclusions. The work is being continued along the same and other lines.

In this investigation it has been assumed, and some evidence presented to support the assumption, that a study of the amino nitrogen content of the developing wheat kernel offers a fairly reliable indirect method of following at least one step in protein synthesis. The value obtained by subtracting the amino nitrogen from the total nitrogen does not represent the nitrogen in the form of protein, for a part of this nitrogen is undoubtedly present in the amino acids in other than amino groups. It is also probable that the protein is not formed instantaneously but is formed in steps, so we would have present various compounds of a polypeptide

nature corresponding to the various reactions in the process of synthesis.

In this paper just one step is under consideration, and conclusions drawn from this step may later be found not to apply to other steps in protein synthesis.

It might appear from the relationship between the moisture and amino nitrogen content of the developing kernel that during the middle period of growth the synthesis of protein in so far as it is related to the change of amino compounds into more complex ones, is regulated by the moisture content of the kernel; and that the decrease of moisture in the kernel is the mechanism which is responsible for protein synthesis. It was found, however, that a decrease in moisture was not necessary for this step in protein synthesis, for the amino nitrogen decreased markedly in the threshed kernels when there was no loss of moisture. This shows that desiccation is not necessary for what is probably the first step in protein synthesis.

It is becoming recognized that in the presence of hydrophylic colloids such as occur in the cell, a part of the water may be held in such a combination by the colloidal substances present that it is not free to act as a solvent; water so combined is frequently spoken of as water of hydration. Newton and Gortner (1922) have applied the term "bound" water to water so held and the term "free" water to that part of the water which is not so firmly held by the colloids and may therefore act as a solvent.

Olson (1923) concludes that the wheat kernel ceases to increase in dry weight after the moisture content of the kernel falls below 40 per cent. The data of Brenchley and Hall (1908-10) indicate a limiting value of 42 to 43 per cent. This may be the moisture content below which there ceases to be free water present which is necessary for the activities of the growing kernel. An examination of Figure 1 indicates that the amino nitrogen ceases to decrease in the developing kernel after the moisture falls below 35 per cent, this value may be taken as another indication of the amount of bound water and is in rough agreement with the moisture content at which an increase in dry weight ceases.

If the amount of bound water is subtracted from the total water content of the kernel, the remainder will represent the amount of free water which can act as a solvent for the amino compounds. As stated previously the points on the middle portion of the curve in Figure 1 fall very nearly on a straight line. It is impossible to determine definitely whether or not this portion of the curve is

actually straight or slightly curved because of experimental error; but if the line is curved, it is only to a slight extent and can be considered as straight without introducing any very considerable error. If we consider this portion of the curve as straight, and subtract from the total water content of the kernel the amount of bound water as represented by the moisture content at which the amino nitrogen ceases to decrease, we can conclude that the concentration of amino compounds in the actual free water of the kernel is constant for this middle period of kernel development. Thus under uniform conditions the following equation will represent approximately the relationship between the amino nitrogen and the water content of the normal developing wheat kernel.

$$\frac{\text{Amino nitrogen}}{\text{Total water—bound water}} = \text{a constant}$$

The samples from which the points in Figure 1 were determined were all collected about 10 o'clock in the morning. The heads were brought to the laboratory and the kernels picked out and thrown into the boiling water by noon. The climatic conditions varied somewhat from day to day. This may partly account for some of the irregularities in Figure 1. Samples collected at sunrise might give a different constant from samples collected at sunset.

The synthesis of protein tends to reduce the concentration of amino nitrogen in the kernel, but an actual decrease in concentration is prevented by the flow of additional material into the kernel from other parts of the plant. Pierre (1865-69), in his monumental investigation of the composition of the wheat plant at various stages of growth, shows that nitrogen decreases in other parts of the plant and increases in the kernel during the period of kernel development. He found the most marked decrease in the leaves.

The fact that the amino nitrogen decreases in the threshed kernels when there is no loss of water indicates that the first part of the complex reaction of protein synthesis is in the main irreversible in the normal plant, and that the mechanism which maintains the concentration of amino compounds constant in the free water of the kernel is not located in the kernel itself. If this is the case there are apparently two ways in which high protein wheat may be formed: (1) the plant yielding high protein wheat may have a more rapid mechanism for changing the transportable forms of nitrogen into the more complex; or (2) a higher concentration of the transportable forms of nitrogen may be maintained in the wheat kernel, thus increasing the rate of reaction according to the law of mass action. A higher concentration of the transportable

forms of nitrogen may be maintained in the water of the wheat kernel in various ways. The actual translocation may be more rapid. Olson (1923) believes that transpiration aids to a greater extent in translocation of nitrogen compounds than of non-nitrogenous material. The nitrogenous materials in the leaves may be changed into the transportable forms more rapidly and thus produce a greater concentration gradient. The most probable explanation is that a higher concentration of the various nitrogen compounds throughout the entire plant may be produced, other things being equal, by a larger amount of available nitrogen in the soil. Several investigators have shown that the protein content of the wheat kernel can be increased by proper nitrogen fertilization. Just what part wheat variety may play it is impossible at present to say.

A comparison of the two curves for Marquis wheat in Figure 1 shows that in general the amino nitrogen was higher in the 1923 samples than in the 1922 samples. The average protein content was 12.1 per cent for the 1922 samples, omitting the first five determinations because the other series did not include such immature samples; and 15.7 for the 1923 samples, showing in this instance that high amino nitrogen in the developing kernel is associated with high protein content. The average protein content of the Kanred wheat was 11.3 per cent, yet apparently the amino nitrogen was higher in the water of the kernel in this case than it was in the 1922 Marquis.

Freezing apparently stops that part of the synthetic reaction which involves the changing of the amino compounds into more complex ones and, if anything, produces a tendency in the reverse direction. This reaction, which is apparently irreversible in the normal kernel, is either stopped by the freezing or changed into a reversible one with a tendency to go in the indirection of forming more amino compounds.

Conclusions

Desiccation is not necessary for the conversion of a part of the amino compounds into more complex ones.

Nitrogen distribution in the immature wheat kernel is greatly influenced by different methods of storage and drying.

Severe freezing has a pronounced effect on the amino nitrogen of the immature wheat kernel. The percentage of amino nitrogen decreases in the normal developing kernel; freezing prevents this decrease and tends to cause an increase.

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COMMENTS ON "GLYCEROL AS AN AID IN ASHING FLOUR"

By

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The use of glycerol to hasten the ashing of flour was proposed in a previous article published in this journal (I, p. 83, 1924). Certain criticisms of the method have come to the attention of the authors, the principal one being that the glycerol-flour mixture tends to boil over the sides of the crucible.

It is believed that the difficulties encountered with the method have arisen more from an insufficiently detailed description of the method than from any existent intrinsic fault in the method itself. The following elaborations are given with a view of indicating more clearly the conditions necessary for the satisfactory application of the method.

It is believed that the efficacy of the glycerol method rests in the formation of a porous char which presents a large reacting surface to the furnace atmosphere. It is also thought that the size and shape of the ashing dish play an important part in the ashing process. A relatively shallow, broad ashing dish permits free access of air to the flour char and thereby favors rapid oxidation. Accordingly crucibles and deep, narrow dishes are not adapted to the purposes of ashing.

The effect of size and shape of the ashing dish and of the physical condition of the flour char on the rate and completeness of ashing, were tested out on 5-gram portions of a clear, hard wheat flour. Untreated and glycerol-alcohol treated portions, respectively, were ashed in dishes with diameters ranging from 3 to 8.5 cm.

and depths from 1.5 to 5 cm. The conditions of incineration were made as nearly identical as possible. The completeness of the ashings was judged by the appearance of the ashes at half-hour intervals.

The ashes of the untreated samples were still very dark after 6 hours. Those in the broad, shallow dishes were incinerated more completely than those in the narrow, deep dishes. The ash of the glycerol-alcohol treated sample in the broadest dish used was entirely white after a period of 2.5 hours; those in the dishes of medium dimensions were a light gray after from 2 to 2.5 hours, and those in the narrow, deep dishes were still dark gray after 5 hours. The tests indicate that broad, shallow dishes are more favorable for rapid ashing than deep, narrow dishes such as crucibles, which prevent or greatly retard satisfactory ashing; and also that the char of glycerol-alcohol treated flour oxidizes more readily to a white or light gray ash than that of untreated flour. It is recommended that, for rapid ashing, the dish be approximately 5 cm. in breadth and 1 to 1.5 cm. in depth.

The inadequate description of the procedure of the previously published method and more experience gained from its use prompt the publication of the following revised description of the method for its more effective application. It is to be noted that 6 cc. instead of 10 cc. of the glycerol-alcohol solution is sufficient for the desired results. No difficulty with overflowing need be expected if dishes of proper dimensions are used. Platinum, porcelain, and silica dishes were found to give identical results.

Method

Apparatus and Reagent

Flat-bottomed ashing dish of platinum, porcelain, or silica; breadth, approximately 5 cm.; depth, 1 to 1.5 cm.

Air-tight desiccator containing re-ignited quicklime or calcium carbide.

Glycerol-alcohol solution made from equal volumes of ash-free redistilled glycerol and 95 per cent alcohol by volume.

Determination

Weigh 5 grams of flour into the ashing dish which previously has been ignited, cooled in the desiccator, and weighed soon after room temperature has been attained. Mix the flour with 6 cc. of the glycerol-alcohol solution and distribute the mixture evenly about the dish. Clean the mixing rod with a small piece of ashless filter paper and add to the dish. Transfer immediately to a furnace

held at approximately 550°C. (dull red) or, if preferred, ignite the alcohol and allow it to burn off before placing in the furnace. Ignite the vapors and leave the furnace door open until flaming ceases. Incinerate to a light gray ash, transfer to the desiccator, and weigh soon after room temperature is attained.

Correct for any blank obtained from the quantity of glycerol-alcohol solution used should no ash-free glycerol be obtainable.

Quantitative data obtained from comparisons of the direct ignition method and the glycerol-alcohol method on six flours are given below. Platinum dishes, diameter 5 cm., depth 1.5 to 2 cm., were used. The ashings under comparison were run in juxtaposition to one another.

It is to be noted that no further consequential loss occurred on continued heating of the untreated samples after approximately 2 to 2.5 hours, altho they were dark and appeared to be incompletely ashed. These tests indicate that the addition of glycerol-alcohol produces a whiter ash than that otherwise obtained, but that the dark ash of untreated samples, which to all appearances is incompletely burned, weighs practically the same as the lighter appearing ash of the proposed method. Apparently the weight of carbon necessary to darken a flour-ash is within the experimental error of the ash method.

Therefore, to determine flour-ash in an untreated sample, it may suffice to continue the process only to constant weight rather than to secure a light gray ash as specified in the A. O. A. C. method. However, for such procedure, it is necessary to reheat and reweigh. This additional time and attention is eliminated by the glycerol-alcohol method in that it produces a white or light gray ash which in itself indicates that the minimum weight has been obtained.

ASH

Soft Wheat Flours

Ashing period	65% Patent			95% Straight			32% Clear		
	Untreated		Glycerol-alcohol Treated	Untreated		Glycerol-alcohol Treated	Untreated		Glycerol-alcohol Treated
	Per cent	Appearance	Per cent Appearance	Per cent	Appearance	Per cent Appearance	Per cent	Appearance	Per cent Appearance
1½ hours	0.372	Very dark	0.368	0.412	Very dark	0.412	0.496	Very dark	0.496
2 hours	0.360	Very dark	0.356	0.400	Very dark	0.404	0.484	Very dark	0.488
2½ hours	0.352	Very dark	0.360	0.396	Very dark	0.400	0.484	Very dark	0.484
3 hours	0.352	Very dark	0.356	0.396	Very dark	0.400	0.484	Very dark	0.484

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Hard Wheat Flours

Ashing period	Short Patent			Long Patent			Clear		
	Untreated		Glycerol-alcohol Treated	Untreated		Glycerol-alcohol Treated	Untreated		Glycerol-alcohol Treated
	Per cent	Appearance	Per cent Appearance	Per cent	Appearance	Per cent Appearance	Per cent	Appearance	Per cent Appearance
1½ hours	0.472	Very dark	0.440	0.472	Very dark	0.460	0.668	Black	0.624
2 hours	0.432	Very dark	0.428	0.460	Very dark	0.460	0.636	Black	0.612
2½ hours	0.428	Very dark	0.424	0.448	Very dark	0.456	0.620	Black	0.608
3 hours	0.432	Very dark	0.432	0.460	Very dark	0.456	0.624	Black	0.612
3½ hours	0.428	Very dark	0.428	0.456	Very dark	0.460	0.620	Black	0.608
8 hours	0.428	Very dark	0.428	0.456	Very dark	0.456	0.616	Black	0.608

CONVERSION TABLES FOR CALCULATING THE ABSORPTION OF FLOUR TO A 13.5 PER CENT MOISTURE BASIS

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The United States government has fixed 13.5% as the maximum moisture content permissible in flour and for obvious reasons the majority of mills endeavor to deliver flour containing this amount. By the time it has reached the baker and he, in turn, has sampled the shipment and forwarded a test sample to a commercial laboratory for analysis, the flour may have dried out considerably. As the percentage of the various constituents of flour (ash, protein, etc.) is affected by variations in the moisture content, it is highly desirable to base the different items of a flour analysis on a definite moisture content and in view of modern milling practice the logical basis to use is 13.5%.

Whenever the data of a flour analysis are expressed on a definite moisture basis, not only the figures for ash, but those for all other constituents should be properly corrected. In no case should absorption be ignored, for it is more affected by a change in moisture than any other figure in the analysis. An obviously simple formula is used to calculate ash, gluten, etc., to a 13.5% moisture basis. However, an entirely different and more complicated formula must be used in calculating absorption.

Two years ago when our laboratory department started to report all analysis data on a 13.5% moisture basis, we developed a formula for calculating absorption, which is identical with the one described by Edward Gookins in the November, 1924, issue of Cereal Chemistry. For calculating absorption to a 13.5% moisture basis, this formula simplifies to the following:

$$\text{Absorption (13.5)} = \left((A + M) \times \frac{86.5}{100 - M} \right) - 13.5$$

Before adopting the formula we verified its correctness to our satisfaction by carefully air-drying a considerable number of samples of flour to various moisture contents. We then determined the absorption experimentally on large samples of the air-dry flour by making regular baking tests. We then determined the moisture content and, calculating the absorption to a 13.5% moisture basis by means of the formula, we obtained close check results.

Owing to the number of samples flowing through our laboratory it was necessary to find a simple means of arriving at these figures. We therefore devised the following conversion tables for calculating absorption to a 13.5% moisture basis.

These tables are accurate to 0.1% which is well within the limits of accuracy in determining absorption, and they contain values of moisture and absorption commonly found. The value for absorption on a 13.5% moisture basis is found at the intersection of a vertical line drawn through the moisture figure and a horizontal line drawn through the figure for absorption as received. The table shows that the change in absorption is about 1.8 times the variation in the moisture content.

TABLE FOR CALCULATING ABSORPTION TO 13.5% MOISTURE
Moisture of flour as received

Absorption as received	9.0	9.1	9.2	9.3	9.4	9.5	9.6	9.7	9.8	9.9
56	48.3	48.4	48.6	48.8	49.0	49.1	49.2	49.4	49.6	49.8
56.5	48.7	48.9	49.1	49.3	49.4	49.6	49.7	49.9	50.1	50.3
57	49.2	49.4	49.6	49.8	49.9	50.1	50.2	50.4	50.5	50.7
57.5	49.7	49.8	50.0	50.2	50.3	50.5	50.6	50.8	51.0	51.2
58	50.2	50.3	50.5	50.6	50.8	51.0	51.1	51.3	51.5	51.7
58.5	50.7	50.8	51.0	51.2	51.3	51.5	51.6	51.8	52.0	52.2
59	51.2	51.3	51.5	51.6	51.8	52.0	52.1	52.3	52.5	52.7
59.5	51.6	51.8	52.0	52.1	52.3	52.5	52.6	52.8	53.0	53.1
60	52.1	52.2	52.4	52.5	52.7	52.9	53.0	53.2	53.4	53.6
60.5	52.6	52.7	52.9	53.0	53.2	53.4	53.5	53.7	53.9	54.1
61	53.1	53.2	53.4	53.5	53.7	53.9	54.0	54.2	54.4	54.5
61.5	53.5	53.7	53.9	54.0	54.2	54.4	54.5	54.7	54.9	55.0
62	54.0	54.2	54.4	54.5	54.7	54.9	55.0	55.2	55.4	55.5
62.5	54.5	54.7	54.9	55.0	55.2	55.3	55.5	55.7	55.9	56.0
63	55.0	55.1	55.3	55.4	55.6	55.8	55.9	56.1	56.2	56.4
63.5	55.4	55.6	55.8	55.9	56.1	56.3	56.4	56.6	56.7	56.9
64	55.9	56.1	56.3	56.4	56.6	56.8	56.9	57.1	57.2	57.4
64.5	56.4	56.6	56.8	56.9	57.1	57.2	57.4	57.6	57.7	57.9
65	56.8	57.0	57.2	57.3	57.5	57.7	57.9	58.1	58.2	58.4
65.5	57.3	57.5	57.7	57.8	58.0	58.2	58.4	58.6	58.7	58.9
66	57.8	58.0	58.2	58.3	58.5	58.7	58.8	59.0	59.2	59.4

TABLE FOR CALCULATING ABSORPTION TO 13.5% MOISTURE—Continued
Moisture of flour as received

Absorption as received	12.0	12.1	12.2	12.3	12.4	12.5	12.6	12.7	12.8	12.9
56	53.3	53.5	53.7	53.9	54.0	54.2	54.4	54.6	54.7	54.9
56.5	53.8	54.0	54.2	54.4	54.5	54.7	54.9	55.1	55.2	55.4
57	54.3	54.5	54.7	54.9	55.0	55.2	55.4	55.5	55.7	55.9
57.5	54.8	55.0	55.2	55.4	55.5	55.7	55.9	56.0	56.2	56.4
58	55.3	55.5	55.7	55.9	56.0	56.2	56.4	56.5	56.7	56.9
58.5	55.8	56.0	56.2	56.3	56.5	56.7	56.9	57.0	57.2	57.4
59	56.3	56.5	56.7	56.8	57.0	57.2	57.4	57.5	57.7	57.9
59.5	56.8	57.0	57.2	57.3	57.5	57.7	57.9	58.0	58.2	58.4
60	57.3	57.5	57.7	57.8	58.0	58.2	58.4	58.5	58.7	58.9
60.5	57.8	58.0	58.2	58.3	58.5	58.7	58.9	59.0	59.2	59.4
61	58.3	58.5	58.7	58.8	59.0	59.2	59.4	59.5	59.7	59.9
61.5	58.8	59.0	59.2	59.3	59.5	59.7	59.9	60.0	60.2	60.4
62	59.3	59.5	59.7	59.8	60.0	60.2	60.4	60.5	60.7	60.9
62.5	59.8	60.0	60.2	60.3	60.5	60.7	60.9	61.0	61.2	61.4
63	60.3	60.4	60.6	60.8	61.0	61.1	61.3	61.5	61.7	61.9
63.5	60.8	60.9	61.1	61.3	61.5	61.6	61.8	62.0	62.2	62.4
64	61.2	61.4	61.6	61.7	61.9	62.1	62.3	62.5	62.6	62.8
64.5	61.7	61.9	62.1	62.2	62.4	62.6	62.8	63.0	63.1	63.3
65	62.2	62.4	62.6	62.7	62.9	63.1	63.3	63.5	63.6	63.8
65.5	62.7	62.9	63.1	63.2	63.4	63.6	63.8	64.0	64.1	64.3
66	63.2	63.4	63.6	63.7	63.9	64.1	64.3	64.5	64.6	64.8

TABLE FOR CALCULATING ABSORPTION TO 13.5% MOISTURE—Continued
Moisture of flour as received

Absorption as received	13.0	13.1	13.2	13.3	13.4	13.5	13.6	13.7	13.8	13.9	14.0
56	55.1	55.3	55.5	55.6	55.8	56.0	56.2	56.4	56.5	56.7	56.9
56.5	55.6	55.8	56.0	56.1	56.3	56.5	56.7	56.9	57.0	57.2	57.4
57	56.1	56.3	56.5	56.6	56.8	57.0	57.2	57.4	57.5	57.7	57.9
57.5	56.6	56.8	57.0	57.1	57.3	57.5	57.7	57.9	58.0	58.2	58.4
58	57.1	57.3	57.5	57.6	57.8	58.0	58.2	58.4	58.5	58.7	58.9
58.5	57.6	57.8	58.0	58.1	58.3	58.5	58.7	58.9	59.0	59.2	59.4
59	58.1	58.3	58.5	58.6	58.8	59.0	59.2	59.4	59.5	59.7	59.9
59.5	58.6	58.8	59.0	59.1	59.3	59.5	59.7	59.9	60.0	60.2	60.4
60	59.1	59.3	59.5	59.6	59.8	60.0	60.2	60.4	60.5	60.7	60.9
60.5	59.6	59.8	60.0	60.1	60.3	60.5	60.7	60.9	61.0	61.2	61.4
61	60.1	60.3	60.5	60.6	60.8	61.0	61.2	61.4	61.5	61.7	61.9
61.5	60.6	60.8	61.0	61.1	61.3	61.5	61.7	61.9	62.0	62.2	62.4
62	61.1	61.3	61.5	61.6	61.8	62.0	62.2	62.4	62.5	62.7	62.9
62.5	61.6	61.8	62.0	62.1	62.3	62.5	62.7	62.9	63.0	63.2	63.4
63	62.1	62.3	62.5	62.6	62.8	63.0	63.2	63.4	63.5	63.7	63.9
63.5	62.6	62.8	63.0	63.1	63.3	63.5	63.7	63.9	64.0	64.2	64.4
64	63.1	63.2	63.4	63.6	63.8	64.0	64.2	64.4	64.6	64.8	65.0
64.5	63.6	63.7	63.9	64.1	64.3	64.5	64.7	64.9	65.1	65.3	65.5
65	64.1	64.2	64.4	64.6	64.8	65.0	65.2	65.4	65.6	65.8	66.0
65.5	64.5	64.7	64.9	65.1	65.3	65.5	65.7	65.9	66.1	66.3	66.5
66	65.0	65.2	65.4	65.6	65.8	66.0	66.2	66.4	66.6	66.8	70.0

TABLE FOR CALCULATING ABSORPTION TO 13.5% MOISTURE—Continued
Moisture of flour as received

Absorption as received	10.0	10.1	10.2	10.3	10.4	10.5	10.6	10.7	10.8	10.9
56	49.9	50.1	50.3	50.4	50.6	50.8	50.9	51.1	51.3	51.4
56.5	50.4	50.6	50.8	50.9	51.1	51.3	51.4	51.6	51.8	51.9
57	50.9	51.0	51.2	51.4	51.6	51.8	51.9	52.1	52.3	52.4
57.5	51.4	51.5	51.7	51.9	52.1	52.3	52.4	52.6	52.8	52.9
58	51.8	52.0	52.2	52.3	52.5	52.7	52.9	53.0	53.2	53.4
58.5	52.3	52.5	52.7	52.8	53.0	53.2	53.4	53.5	53.7	53.9
59	52.8	53.0	53.2	53.3	53.5	53.7	53.8	54.0	54.2	54.3
59.5	53.3	53.5	53.7	53.8	54.0	54.2	54.3	54.5	54.7	54.8
60	53.8	53.9	54.1	54.3	54.4	54.6	54.8	55.0	55.2	55.3
60.5	54.3	54.4	54.6	54.7	54.9	55.1	55.3	55.5	55.6	55.8
61	54.7	54.9	55.1	55.2	55.4	55.6	55.8	56.0	56.1	56.3
61.5	55.2	55.4	55.6	55.7	55.9	56.1	56.3	56.5	56.6	56.8
62	55.7	55.9	56.1	56.2	56.4	56.6	56.7	56.9	57.1	57.2
62.5	56.2	56.4	56.6	56.7	56.9	57.1	57.2	57.4	57.6	57.7
63	56.7	56.8	57.0	57.2	57.3	57.5	57.7	57.9	58.0	58.2
63.5	57.1	57.3	57.5	57.6	57.8	58.0	58.2	58.4	58.5	58.7
64	57.6	57.8	58.0	58.1	58.3	58.5	58.7	58.9	59.0	59.2
64.5	58.1	58.3	58.5	58.6	58.8	59.0	59.2	59.4	59.5	59.7
65	58.6	58.8	59.0	59.1	59.3	59.5	59.7	59.9	60.0	60.2
65.5	59.1	59.3	59.5	59.6	59.8	60.0	60.1	60.3	60.5	60.6
66	59.5	59.7	59.9	60.0	60.2	60.4	60.6	60.8	60.9	61.1

TABLE FOR CALCULATING ABSORPTION TO 13.5% MOISTURE—Continued
Moisture of flour as received

Absorption as received	11.0	11.1	11.2	11.3	11.4	11.5	11.6	11.7	11.8	11.9
56	51.6	51.8	52.0	52.2	52.3	52.5	52.7	52.9	53.0	53.2
56.5	52.1	52.3	52.5	52.6	52.8	53.0	53.2	53.4	53.5	53.7
57	52.6	52.8	53.0	53.1	53.3	53.5	53.7	53.8	54.0	54.2
57.5	53.1	53.3	53.5	53.6	53.8	54.0	54.2	54.3	54.5	54.7
58	53.6	53.8	54.0	54.1	54.3	54.4	54.6	54.7	54.9	55.1
58.5	54.1	54.3	54.5	54.6	54.8	55.0	55.2	55.3	55.5	55.7
59	54.5	54.7	54.9	55.0	55.2	55.4	55.6	55.7	55.9	56.1
59.5	55.0	55.2	55.4	55.5	55.7	55.9	56.1	56.2	56.4	56.6
60	55.5	55.7	55.9	56.0	56.2	56.4	56.6	56.7	56.9	57.1
60.5	56.0	56.2	56.4	56.5	56.7	56.9	57.1	57.2	57.4	57.6
61	56.5	56.7	56.9	57.0	57.2	57.4	57.6	57.7	57.9	58.1
61.5	57.0	57.2	57.4	57.5	57.7	57.9	58.1	58.2	58.4	58.6
62	57.4	57.6	57.8	58.0	58.2	58.4	58.6	58.7	58.9	59.1
62.5	57.9	58.1	58.3	58.5	58.7	58.9	59.1	59.2	59.4	59.6
63	58.4	58.6	58.8	59.0	59.1	59.3	59.5	59.6	59.8	60.0
63.5	58.9	59.1	59.3	59.5	59.6	59.8	60.0	60.1	60.3	60.5
64	59.4	59.6	59.8	60.0	60.1	60.3	60.5	60.6	60.8	61.0
64.5	59.9	60.1	60.3	60.5	60.6	60.8	61.0	61.1	61.3	61.5
65	60.4	60.6	60.8	61.0	61.1	61.3	61.5	61.6	61.8	62.0
65.5	60.8	61.0	61.2	61.4	61.6	61.8	62.0	62.1	62.3	62.5
66	61.3	61.5	61.7	61.9	62.0	62.2	62.4	62.6	62.8	63.0

SUMMARY OF THE ACTIVITIES OF THE A. O. A. C. FOR 1924 ON STUDIES OF METHODS FOR THE ANALYSIS OF CEREAL FOODS¹

Prepared by Raymond Hertwig, U. S. Department of Agriculture,
Bureau of Chemistry, Washington, D. C.

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The Association of Official Agricultural Chemists at its 1923 meeting designated a general referee and four associate referees for the study of methods of analysis of cereal foods. The studies undertaken were limited to wheat flour analyses.

A summary is given below of the report on cereal foods made by the general referee, Raymond Hertwig, of the Bureau of Chemistry, and read at the October meeting of the association. The report includes a discussion of the studies of the associate referees and the recommendations for action by the association.

Attention is called to the desirability in certain instances of having two types of methods for the analysis of food products. First, umpire methods of analysis whose chief merit is high accuracy. When such methods are not economical of time and cost, then effort should be made by the association to devise and develop the second type of methods, namely, routine methods for economy of time and cost but whose limits of accuracy may be somewhat wider than those of the umpire methods, altho the results must be sufficiently accurate for their acceptance for the practical needs of the majority of chemists. The umpire methods serve to standardize the routine methods and to check any results by the routine methods which may be open to question.

The study of methods for the sampling of flour and the preparation of the sample for analysis was assigned to associate referee G. J. Morton, of the San Francisco Station, Bureau of Chemistry. This associate referee was unable to submit a report in time for reading at the association meeting. In lieu thereof, the general referee discussed the general principles of sampling flour. He emphasized the importance of obtaining representative samples of flour in storage, as the dependability of both physical and chemical examination of the product rests fundamentally upon the sample submitted for analysis. The most painstaking, accurate analysis is vitiated by a nonrepresentative sample. It is of especial importance when the moisture content of flour lots is under investigation.

¹ Report presented by Mr. Hertwig at the annual convention of the Association of Official Agricultural Chemists on October 21, 1924, at Washington, D. C. The complete report will be published in due course in the journal of the A. O. A. C.

Sacked flour in storage presents a very difficult problem in obtaining a representative sample, especially with respect to the moisture content of the entire lot. The surfaces of the sacks are irregularly exposed, consequently moisture changes are unequally distributed about the flour mass of the respective sacks. These irregular moisture changes are incapable of mathematical expression and therefore no sampling instrument can be so devised with dimensions calculated from mathematical considerations as to withdraw a representative sample from sacks of flour in storage. A representative sample can at best be only approximated and no simple procedure can be recommended for taking a representative sample of flour in storage, as conditions encountered differ so widely. A recommendation is made for the adoption of certain general directions for the taking of samples of flour in storage and for the preparation of the samples for analysis. Further recommendation is made for continuing the study of this subject during the coming year, bearing in mind the addition of any practical details to the directions proposed which may be of assistance in the sampling procedure, and also to considerations of the type of sampler to be used and the proportionate number of sacks to be sampled of lots of flour in storage.

Methods for the determination of moisture in flour were studied by associate referee, G. C. Spencer, of the Bureau of Chemistry. The loss in weight which occurs when a flour sample is subjected to varying pressures and temperatures, under temperatures at which detectable decomposition sets in, is a function of both the temperature and pressure conditions. The absolute moisture content of the flour is a limit value which can be only approached, not actually reached, under usual working conditions. The loss in weight is a definite quantity only when the conditions of pressure and temperature are fixed.

Associate referee Spencer gave special study to the vacuum method for the determination of moisture. He recommends a vacuum method as an umpire method for this determination. A temperature of 100°C. and a partial vacuum with a definite pressure of 25 mm. of mercury are specified in this method. The sample dishes are left loosely covered during the drying period. The method as described by the general referee in his report is recommended for adoption as the official umpire method for determining moisture.

The umpire vacuum method just mentioned lacks economy of time and cost, consequently associate referee Spencer devised a

rapid, practical, routine method for determining moisture in flour which is capable of giving results closely approximating those of the umpire vacuum method. This rapid method is especially proposed for flour moisture determinations in mill laboratories, for routine governmental regulatory work, for the usual analyses in commercial considerations, and in ordinary investigational work. The routine method specifies the drying of approximately two grams of sample for a period of one hour at a temperature of 130°C . The method as described in the recommendations of the general referee is recommended for adoption as a tentative routine method.

The general referee, by way of discussion of the two above mentioned methods for moisture in flour, mentions that the methods determine the definite loss in weight a flour sample undergoes under certain definite conditions. The fact that the weight loss is constant under the specified conditions makes these two methods practical and usable for all considerations excepting the determination of the absolute moisture content of flour. Past methods for moisture determination in flour, on the other hand, are not practical, as they do not always yield the same results. The physical and chemical relationship of the moisture loss to the flour constituents is not considered in these methods, nor is such consideration of practical import. Scientific knowledge of this relationship at this time is not definite. For practical purposes, the loss in weight determined by the two methods may be looked upon as the external water phase held by surface attraction of the flour particles. Irrespective of how the moisture determined by the methods is related to the flour substances, the general referee emphasizes that the methods have their chief value in that they always yield like results under the specified conditions and that the results are as near the absolute as are practically obtainable. From these viewpoints, the methods meet the actual and practical purposes of association chemists as well as of outside chemists.

Associate referee Spencer found that the present official hydrogen drying method for determining moisture in flour was unreliable. Recommendation is made that this method be dropped as an association method.

The general referee recommends that the routine method for moisture in flour be further studied during the coming year with a view to its adoption as an official method.

C. E. Mangels, of the North Dakota Agricultural Experiment Station, as associate referee on the study of methods for determin-

ing ash in flour, submitted a modification of the present official method of the association which was proposed by C. H. Bailey, acting as referee in 1922, and a glycerol method devised by Raymond Hertwig and L. H. Bailey, of the Bureau of Chemistry, to collaborative study. Mr. Mangels obtained satisfactory collaborative results by the two methods and recommends the modified official method for adoption. The associate referee himself studied a method in which calcium acetate is added to the flour to prevent fusing of the ash and enabling the use of higher temperatures than the regular ash method. He recommends further study of the glycerol method and of the calcium acetate method.

Raymond Hertwig and L. H. Bailey have found that the addition of fine asbestos fiber to a flour very markedly shortens the ashing period. In their experience, this procedure yields an ash very slightly lower in quantity than that obtained by the regular ashing procedure. By this procedure, a white ash is obtained in about forty-five minutes. The unusual rapidity of the incineration and the simplicity of the procedure are such as to deserve further study.

The general referee recommends for adoption as official the method for the determination of ash in flour as described in the recommendations of his report and also that the present official method for this determination be dropped. The method recommended specifies the approximate temperature for the incineration and other details of the procedure. He also recommends that the glycerol method, the calcium acetate method, and the asbestos method for the rapid determination of ash in flour be studied during the coming year with a view to the development of a method especially adapted to routine work requiring economy of time.

Paul F. Sharp, of the Montana Agricultural Experiment Station, was appointed by the association to study methods for determining glutenin in flour. This associate referee has been carrying on an excellent research on a study of means for determining this substance, but, owing to the large amount of experimentation necessary, he was not able to present to this meeting anything more than a progress report.

The general referee recommends the continuance of the study of methods for the determination of glutenin in flour by the present associate referee and that, in addition, he be requested to include in his investigations any possible modifications of the present tentative methods for "alcohol soluble protein" and "protein soluble in five per cent potassium sulphate solution" which his work may indicate as imperative.

The general referee proposes a method devised by himself for the determination of fat in flour by an acid digestion method. This method extracts fat-like substances from flour more completely than the official direct ether extraction method. The fat determined by this acid digestion method probably consists essentially of the true fats, fatty acids, unsaponifiable matter, and sterols. Such substances as lecithin are destroyed by the acid hydrolysis. The general referee recommends the adoption of this method as tentative for the examination of flour.

A method for the determination of lipoids and lipid phosphoric acids (P_2O_5), devised by Raymond Hertwig, is proposed for adoption as a tentative method for flour analysis. This method extracts more ether-soluble substances from flour than any other known method and includes lecithin and allied substances along with true fats, fatty acids, unsaponifiable matter, coloring matter, sterols, etc.

A method for the determination of "water-soluble protein-nitrogen precipitable by 40 per cent alcohol" devised by Raymond Hertwig to determine essentially the albumen of flours, is recommended for adoption as a tentative method of flour examination.

Associate referee Armin Seidenberg studied various methods for the determination of chlorine in bleached flour. He proposes the development of a method which would give negative results for unbleached flours and positive results for chlorine bleached flours only. He was unable to develop the method to a state at which he could offer it to collaborative study, but apparently developed the fundamentals for such a method. The general referee recommends that study of the method developed by this associate referee be continued.

The general referee recommends that studies be made of methods of analysis of bread, and that in this connection effort be made to apply association methods for wheat flour and alimentary pastes so far as they are practical. He also recommends that the determination of moisture in alimentary paste be further studied with a view to its determination by the two methods recommended for adoption for determining moisture in flour in his report.

**REPORT OF SUB-COMMITTEE C OF THE A. O. A. C. ON
RECOMMENDATIONS OF THE REFEREE
ON CEREAL FOODS**

It is recommended:

(1) That the referee study methods for taking samples of flour and preparing them for analysis.

Approved.

(2) That the official method for the determination of moisture in flour be amended by deleting the phrase "or in a current of hydrogen or."

Approved.

(3) That the vacuum method described by the referee in his report¹ for the determination of moisture in flour be subjected to further collaborative study.

Approved.

(4) That the rapid method for determining moisture in flour as described by the referee in his report be subjected to further study during the coming year.

Approved.

(5) That the method described by the referee in his report¹ for the determination of ash in flour be adopted as an official method. (Final action).

Approved.

(6) That Section 22 "Ash," under the heading "Wheat Flour," Chapter XIV "Cereal Foods," be deleted.

Approved.

(7) That the rapid methods described by the referee in his report¹ for determining ash in flour be subjected to further studies during the coming year.

Approved.

(8) That the referee study methods for the determination of glutenin in flour during the coming year.

Approved.

(9) That the methods "Fat (Acid Hydrolysis Method)" and "Lipoids and Lipoid Phosphoric Acid (P_2O_5)" described by the referee in his report² for the examination of flour, be subjected to collaborative study.

Approved.

(10) That the method described by the referee in his report⁴

¹ Jour. Assoc. Official Agr. Chemists, 1925

² Assoc. Official Agr. Chemists, Methods 1920, 167.

³ Jour. Assoc. Official Agr. Chemists, 1924.

⁴ Ibid.

for the determination "Water Soluble Protein Nitrogen Precipitable by 40% Alcohol," be subjected to collaborative study.

Approved.

(11) That the method submitted by the associate referee for the determination of chlorine in bleached flours be studied during the coming year.

Approved.

(12) That studies be undertaken of methods for the examination of bread, which studies shall include the association methods for wheat flour and alimentary pastes, in so far as same are practical.

Approved.

(13) That the determination of moisture in alimentary pastes be further studied.

Approved.

BOOK REVIEW

Modern Cereal Chemistry, by D. W. Kent-Jones

Published by the Northern Publishing Co., Liverpool, England.
1924. Price, 25 Shillings

This useful book should be welcomed by cereal chemists, as no comprehensive survey of contemporary work in this field of applied science has been published during recent years. The substantial developments in cereal chemistry, particularly those of the last decade, have been presented in readable form by the author. The scope of the work is well indicated by the chapter headings, which are as follows:

I. Composition of wheat flour and classes of wheat. II. Vitamins and flour. III. Colloidal chemistry. IV. Hydrogen-ion concentration. V. Colloidal chemistry of flours and the baking process. VI. Composition of mill products. VII. Bleaching and flour improvers. VIII. Conditioning. IX. Moisture in wheat and flour. X. Analysis of flour, etc.

As might be anticipated, Kent-Jones devotes more space to such subjects as flour improvers, and less to such subjects as wheat characteristics and qualities, than would be the case if an American rather than an English chemist, were the author. His discussion of the nutritive value of wheat products is devoted largely to a consideration of the vitamins, and is not balanced with a treat-

ment of other nutritional factors. In certain other departments of the book, assertions are not invariably accompanied by the data, which would establish them as facts. A real service has been rendered those who are engaged in control or research work in the cereal industries, however, by the inclusion of the results of numerous researches. The discussion of the fundamentals of the significance and determination of hydrogen-ion concentration and the consideration of the colloidal state of matter as it pertains to milling and baking, will appeal to many who have been looking for a concise survey of these important subjects.

C. H. BAILEY.

NOTE

A catalog issued by the Thermo Electric Instrument Company has recently appeared, in which type R and type HT ovens, manufactured by this firm, are described. Certain improvements in the well known Freas thermo regulators have been incorporated, which insure a continued alignment of the contact points. Expiration of patents covering the use of nickel chromium alloys now makes it possible to use nichrome wire in their heating elements.

SUSTAINING MEMBERS OF AMERICAN ASSOCIATION
OF CEREAL CHEMISTS

Arkansas City Milling Co., Arkansas City, Kan.
Bakeries Service Corporation, Chicago, Ill.
Banks, A. J., Ogilvie Flour Milling Company, Montreal, Canada.
Dunwoody Industrial Institute, Minneapolis, Minn.
El Reno Mill & Elevator Co., El Reno, Okla.
Gooch Milling & Elevator Co., Lincoln, Neb.
W. W. Hatton, c/o Messrs. Sale and Frazer, Tokio, Japan
Hecker-Jones-Jewell Milling Co., New York City, N. Y.
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The Hoffman Mills, Enterprise, Kan.
The Ismert-Hincke Milling Company, Kansas City, Mo.
International Milling Co., Minneapolis, Minn.
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Larabee Flour Mills Corporation, Kansas City, Mo.
John J. Lentz, Williams Bakery, Scranton, Pa.
Liberty Yeast Corporation, New York City.
Milton-Hersey Co., Ltd., Winnipeg, Canada.
Minnesota State Experimental Flour Mill, Minneapolis, Minn.
Montana Experiment Station Grain Laboratory, Bozeman, Mont.
V. H. Noury & Van der Lande (Novadel Processes), Buffalo, N. Y.
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Pillsbury Flour Mills Company, Minneapolis, Minn.
Portland Flour Mills Co., Portland, Ore.
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Societe Anonyme de Minoteries et D'Elevateurs A Grains, 24 Rue Royale,
Bruxelles, Belgium
Southwestern Milling Co., Kansas City, Mo.
Bernard Stern & Sons, Inc., Milwaukee, Wis.
Wallace & Tiernan Co., Newark, N. J.
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